UNIVERSITI PUTRA MALAYSIA

IRON BIOAVAILABILITY FROM SPIRULINA (ARTHROSPIRA PLATENSIS) AND ITS INTERACTIONS WITH OTHER DIETARY FACTORS IN VITRO AND IN VIVO

LOH SU PENG

FPSK(P) 2004 1
IRON BIOAVAILABILITY FROM SPIRULINA (ARTHROSPIRA PLATENSIS) AND ITS INTERACTIONS WITH OTHER DIETARY FACTORS IN VITRO AND IN VIVO

By

LOH SU PENG

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

June 2004
I lift up my eyes to the hills
Where does my help come from?
My help comes from the LORD
the Maker of heaven and earth

*Psalm 121:1-2*
Deficiency of iron is common worldwide. Various approaches have been used to improve iron intake and absorption. These include the use of spirulina, a microalgae that is already popular in many Asian countries as a functional food supplement. The main objective of this study was to determine the iron bioavailability from spirulina and its interactions with other dietary factors both in vitro and in vivo.

In vitro digestion/Caco-2 cell culture system accompanied by either centrifugation or dialysis step was used to assess the availability of iron from spirulina. Using the centrifugation method, the cultured and commercial spirulina yielded significantly higher results (P< 0.05) than then dialysis method, both in the form of iron available for uptake and the actual amount of iron being transported across the Caco-2 cells. The amount of available iron and iron being transported from ferrous sulphate (FeSO₄) did not differ significantly for both the dialysis and centrifugation method. The effects of
different molar ratios of nutrients (calcium, ascorbic acid, zinc, tannic acid and caffeine) to iron on the availability of iron from cultured spirulina differs in comparison with FeSO₄. In the presence of lower concentrations of calcium (1:5, 1:10, 1:15 and 1:20 Fe:Ca molar ratios), iron from spirulina was not significantly inhibited compared to FeSO₄ but at higher concentrations (1:37.34, 1:74.67 and 1:149.34 Fe:Ca molar ratios) iron from both spirulina and FeSO₄ was significantly inhibited. The availability of iron from spirulina in the presence of ascorbic acid were not significantly enhanced at all the molar ratios tested (1:0.5, 1:1, 1:1.5 and 1:2 Fe:AA molar ratios) whereas iron availability from FeSO₄ were significantly higher for all the molar ratios. Both zinc and tannic acid were more inhibiting on iron availability from spirulina in comparison to FeSO₄. As for caffeine, it did not show any significant inhibitory effects on both iron availability from spirulina and FeSO₄. Two iron pools could coexist in the spirulina, one containing organic iron and another comprising inorganic iron. Organic iron is known to be more bioavailable and less affected by the presence of other nutrients. This could be one of the explanations why the iron from this algae is highly available and its bioavailability is not significantly affected by other nutrients as in FeSO₄.

Haemoglobin repletion assay was used to further investigate the effect of calcium on absorption of iron in spirulina and it comparison with FeSO₄. In this study, haemoglobin and haematocrit levels of male Sprague-Dawley rats fed both spirulina and FeSO₄ were found similar although the dose of FeSO₄ used had twice the amount of iron compared to that in spirulina. The
presence of calcium did not significantly reduced the haematological value in rats fed spirulina and FeSO$_4$. The percentage of haemoglobin regeneration efficiency (HRE) obtained was significantly higher in rats fed spirulina compared with rats fed FeSO$_4$ indicated that the absorption efficiency were better from iron in spirulina compared to iron in FeSO$_4$.

The distribution study of iron from spirulina and FeSO$_4$ in the presence of calcium was done using iron deficient and iron normal male ICR mice fed either spirulina or FeSO$_4$ tagged extrinsically with $^{59}$Fe. The amount of $^{59}$Fe being absorbed by the iron deficient mice fed spirulina was comparable with those fed FeSO$_4$ at 6 h and 24 h. However at 7 d, the FeSO$_4$ group showed better absorption than the spirulina group. In the iron normal mice, a significantly lower percentage of $^{59}$Fe was observed in mice fed spirulina compared to mice fed FeSO$_4$ at 6 h and 24 h indicating that iron from spirulina were not readily absorbed in iron normal states, which could prevent iron overload and toxicity. The presence of calcium did not significantly inhibit iron absorption in spirulina as shown in the in vitro study.

This study indicated that spirulina is a concentrated source of iron for both supplementation and fortification. Iron from spirulina is highly bioavailable and easily absorbed by the body especially in the iron deficient state. Beside providing the necessary iron, it could also prevent iron overload and toxicity in normal iron status and thus making spirulina suitable for both the iron deficient and normal iron status.
Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

BIOAVAILABILITI FERUM DARIPADA SPIRULINA (ARTHROSPIRA PLATENSIS) DAN INTERAKSINYA DENGAN FAKTOR DIETARI LAIN SECARA IN VITRO DAN IN VIVO

Oleh
Loh Su Peng
Jun 2004

Pengerusi : Profesor Madya Maznah Ismail, Ph.D.
Fakulti : Perubatan dan Sains Kesihatan


Gabungan proses penghadaman in vitro dan sel kultur Caco-2 yang diikuti samada dengan langkah emparan atau dialisis telah digunakan untuk menilai keperolehan (availability) ferum daripada spirulina. Dengan menggunakan kaedah emparan, spirulina yang dikultur dan spirulina komersial telah memberikan hasil yang lebih signifikan (P<0.05) berbanding dengan kaedah dialisis dari segi bentuk ferum yang tersedia untuk penyerapan dan juga jumlah sebenar ferum yang diangkut melalui sel Caco-
Asai haemoglobin repletion telah digunakan untuk mengkaji secara in vivo kesan kalsium terhadap penyerapan ferum daripada spirulina dan perbandingannya dengan FeSO₄. Dalam kajian ini, didapati hemoglobin, hematokrit dan paras mean corpuscular volume (MCV) tikus jantan Sprague-Dawley yang diberi diet spirulina dan FeSO₄ adalah serupa walaupun dos ferum daripada FeSO₄ adalah dua kali ganda lebih tinggi dari jumlah ferum spirulina. Kehadiran kalsium tidak merencat secara signifikan nilai hematologi tikus yang diberi spirulina tetapi tidak dalam FeSO₄. Peratusan "kecekapan pembaharuan hemoglobin" (haemoglobin repletion efficiency) yang didapati adalah lebih tinggi secara signikan dalam tikus yang diberi spirulina berbanding tikus yang diberi FeSO₄. Ini menunjukkan kecekapan penyerapan ferum daripada spirulina adalah lebih baik jumlah ferum daripada FeSO₄.

Kajian penyebaran ferum daripada spirulina dan FeSO₄ dengan kehadiran kalsium telah dijalankan dengan menggunakan mencit jantan ICR yang kekurangan ferum dan normal ferum. Mereka diberi sama ada diet spirulina atau FeSO₄ yang telah dilabel ⁵⁹Fe secara ekstrinsik. Jumlah ⁵⁹Fe yang diserap oleh mencit kekurangan ferum adalah serupa antara kumpulan yang diberi spirulina dengan kumpulan yang diberi FeSO₄ pada jam ke-6 dan ke-24. Walau bagaimanapun, pada hari ke-7, kumpulan FeSO₄ menunjukkan penyerapan yang lebih baik berbanding dengan kumpulan spirulina. Dalam mencit normal ferum, peratus ⁵⁹Fe yang lebih rendah dalam spirulina berbanding dengan FeSO₄ pada jam ke-6 dan ke-24 menunjukkan ferum dari spirulina tidak dapat tersedia untuk diserap yang mana dapat

Keseluruhan kajian ini menunjukkan spirulina merupakan sumber ferum yang berkepekatan tinggi untuk dijadikan suplemen dan fortifikasi makanan. Bioavailabiliti ferum dari spirulina adalah tinggi dan mudah diserap oleh tubuh terutamanya dalam keadaan kekurangan ferum. Selain dapat memberi ferum yang diperlukan, ia juga dapat menghalang berlakunya kesaratan ferum dan ketoksikan dalam keadaan normal ferum dan oleh itu spirulina adalah sesuai untuk kedua-dua keadaan kekurangan ferum dan normal ferum.
ACKNOWLEDGEMENTS

A journey is easier when you travel together. Interdependence is certainly more valuable than independence. I have been immensely fortunate to have a phenomenal group of people nurturing my research and me and this thesis would not be complete without recognising their efforts.

I acknowledge, first and foremost, my dependence on God. I want to thank Him, who has sustained me through these, the best and toughest years of my life, providing for all my needs, giving me the strength to study and finally granting me the ability to finish this thesis. He ordered my steps in every aspect of this study, and, as with all of my life. I recognize my utter reliance on Him. I thank God for the marvelous ways He has brought people into my life that have helped me achieve my goals.

I am deeply indebted to my supervisor Assoc Prof Dr Maznah Ismail without whom this thesis would not be possible. Despite the pressure of work, she has devoted time and effort to teach me both in this research and writing it that my labours will never be able to match. My special gratitude also goes to all my co-supervisors, Prof Abdul Salam Abdullah, Dr Rehir Dahalan and Dr Hishamuddin Omar whose guidance, suggestion and vast experiences, have assisted me in the completion of this thesis.

I would like to acknowledge all the lecturers and staff of the Department of Nutrition and Health Sciences specifically and Faculty of
Medicine and Health Sciences in general for providing all the necessary support and help during various stages of the thesis preparation. Equally I wish to thank my fellow lab mates for helping out when I was in need. I would also like to thank the staff of the Medical Technology Division, MINT for making the research in MINT possible.

My thanks will not be complete if I did not mention my church members and friends - your prayer and encouragement kept me focused and sustained me through. Peck Choo, thank you for being so meticulous in the editorial work.

Everything that I have accomplished in life can be traced to having a loving and supportive family. Papa, mummy, Su Ling, Thiam Choy and Ah Keong, all your contributions to this work are immeasurable. Last but certainly not the least, I am forever indebted to the understanding, help and love shown by my husband, Liang Kwong.
I certify that an Examination Committee met on 2\textsuperscript{nd} June 2004 to conduct the final examination of Loh Su Peng on her Doctor of Philosophy thesis entitled "Iron Bioavailability from Spirulina (Arthrospira platensis) and its Interactions with Other Dietary Factors in vitro and in vivo" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

**Khor Geok Lin, Ph.D.**  
Professor  
Faculty of Medicine and Health Science  
Universiti Putra Malaysia  
(Chairman)

**Maznah Ismail, Ph.D.**  
Associate Professor  
Faculty of Medicine and Health Science  
Universiti Putra Malaysia  
(Member)

**Dato' Abdul Salam Abdullah, Ph.D.**  
Professor  
Faculty of Veterinary Medicine  
Universiti Putra Malaysia  
(Member)

**Hishamuddin Omar, Ph.D.**  
Faculty of Science and Environmental Studies  
Universiti Putra Malaysia  
(Member)

**Rehir Dahalan, Ph.D.**  
Atomic Energy Licensing Board  
(Member)

**Dennis D. Miller, Ph.D.**  
Professor  
Department of Food Science  
Cornell University  
(Independent Examiner)

\[\text{Signature}\]

**GULAM RUSUL RAHMAT ALI, Ph.D.**  
Professor/Deputy Dean  
School of Graduate Studies  
Universiti Putra Malaysia  

Date: 26 AUG 2004
The thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirements for the degree of Doctor of Philosophy. The members of the Supervisory Committee are as follows:

**Maznah Ismail, Ph.D.**  
Associate Professor  
Faculty of Medicine and Health Sciences  
Universiti Putra Malaysia  
(Chairman)

**Dato' Abdul Salam Abdullah, Ph.D.**  
Professor  
Faculty of Veterinary Medicine  
Universiti Putra Malaysia  
(Member)

**Hishamuddin Omar, Ph.D.**  
Faculty of Science and Environmental Studies  
Universiti Putra Malaysia  
(Member)

**Rehir Dahalan, Ph.D.**  
Atomic Energy Licensing Board  
(Member)

---

**AINI IDERIS, Ph.D.**  
Professor/Dean  
School of Graduate Studies  
Universiti Putra Malaysia  

**Date:** 20 SEP 2004
DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

LORISU PENG

Date: 25.09.04
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABSTRACT</td>
<td></td>
<td>iii</td>
</tr>
<tr>
<td>ABSTRAK</td>
<td></td>
<td>vi</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>APPROVAL</td>
<td></td>
<td>xii</td>
</tr>
<tr>
<td>DECLARATION</td>
<td></td>
<td>xiv</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td></td>
<td>xviii</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td></td>
<td>xxi</td>
</tr>
</tbody>
</table>

## CHAPTER

### 1 INTRODUCTION
1.1 Objective
   1.1.1 General objective
   1.1.2 Specific objectives

### 2 LITERATURE REVIEW
2.1 Iron functions and chemistry
2.2 Iron absorption and metabolism
2.3 Factors influencing iron absorption
   2.3.1 Luminal factors
   2.3.2 Dietary factors
   2.3.3 Physiological factors
2.4 Mechanism of iron absorption
   2.4.1 Mucosal uptake of iron from the lumen
   2.4.2 Movement of iron within the intestinal cell
   2.4.3 Transfer of iron from the cell to the circulation
2.5 Measurement of iron absorption
   2.5.1 In vitro methods
   2.5.2 In vivo methods
2.6 Arthrospira platensis (Spirulina)
   2.6.1 Introduction
   2.6.2 Morphology and Taxonomy
   2.6.3 Nutritional Value of Spirulina
   2.6.4 Health benefits of Spirulina

### 3 THE EFFECTS OF VARIOUS DIETARY FACTORS ON THE BIOAVAILABILITY OF IRON FROM ARTHROSPIRA PLATENSIS (SPIRULINA) IN VITRO
3.1 Introduction
3.2 Materials and methods
   3.2.1 Materials
3.2.2 Methods 65
3.2.3 Statistical analysis 78
3.3 Results 79
  3.3.1 Mineral content 79
  3.3.2 Confluency assay 80
  3.3.3 Morphological aspects of Caco-2 cell differentiation 83
  3.3.4 Protein concentration 85
  3.3.5 Iron displacement from *Spirulina* 87
  3.3.6 Iron bioavailability from *Spirulina* using two different in vitro methods 90
  3.3.7 Effect of dietary factors on iron bioavailability from *Spirulina* 93
3.4 Discussion 111

4 THE EFFECTS OF CALCIUM SUPPLEMENTATION ON THE BIOAVAILABILITY OF IRON FROM *ARTHROSPIRA PLATENSIS* (SPIRULINA) IN VIVO
  4.1 Introduction 122
  4.2 Materials and methods 124
    4.2.1 Animals and diets 124
    4.2.2 Hemoglobin repletion assay 126
    4.2.3 Iron content in liver and spleen 129
    4.2.4 Statistical analysis 129
  4.3 Result 130
    4.3.1 Food intake evaluation and growth 130
    4.3.2 Hematological variables 132
    4.3.3 Iron content of liver and spleen 138
  4.4 Discussion 140

5 THE EFFECTS OF CALCIUM SUPPLEMENTATION ON IRON DISTRIBUTION FROM FeSO₄ AND *ARTHROSPIRA PLATENSIS* (SPIRULINA) IN VIVO
  5.1 Introduction 146
  5.2 Materials and methods 147
    5.2.1 Animals and diets 147
    5.2.2 Establishment of iron status 147
    5.2.3 Experimental design 148
    5.2.4 Determination of haematological parameter 151
    5.2.5 ⁵⁹Fe analyses 152
    5.2.6 Statistical analysis 153
  5.3 Result 153
    5.3.1 Growth and Iron status 153
    5.3.2 Standardization of μCi and count per minute (cpm) 159
    5.3.3 ⁵⁹Fe uptake and distribution 160
  5.4 Discussion 169
6 GENERAL DISCUSSION AND CONCLUSION
6.1 Recommendations for further studies
6.2 Recommendations for public health nutrition and consumer health promotion

BIBLIOGRAPHY
BIODATA OF THE AUTHOR
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1 Host related factors that affect iron absorption</td>
<td>25</td>
</tr>
<tr>
<td>2.2 Techniques used to study iron bioavailability</td>
<td>34</td>
</tr>
<tr>
<td>2.3 Definitions of absorption</td>
<td>42</td>
</tr>
<tr>
<td>2.4 Advantages and disadvantages of using stable isotopes</td>
<td>44</td>
</tr>
<tr>
<td>2.5 Proximate composition (% dry weight) of <em>A. platensis</em>, <em>A. maxima</em> and soy bean meal</td>
<td>53</td>
</tr>
<tr>
<td>2.6 Distribution of fatty acids in two strains of spirulina namely <em>A. maxima</em> and <em>A. platensis</em></td>
<td>55</td>
</tr>
<tr>
<td>2.7 Vitamins content of spirulina</td>
<td>57</td>
</tr>
<tr>
<td>2.8 Mineral content (range mg/kg dry weight) in spirulina</td>
<td>58</td>
</tr>
<tr>
<td>3.1 Tissue dehydration for transmission electron microscopy</td>
<td>69</td>
</tr>
<tr>
<td>3.2 Tissue infiltration with resin and acetone mixture</td>
<td>69</td>
</tr>
<tr>
<td>3.3 Various amount of protein used for a standard curve</td>
<td>71</td>
</tr>
<tr>
<td>3.4 Ratios of Fe to nutrients used for experiment</td>
<td>78</td>
</tr>
<tr>
<td>3.5 Mineral composition of cultured spirulina and commercial spirulina</td>
<td>79</td>
</tr>
<tr>
<td>3.6 The absorbance and protein concentration values for Caco-2 cells grown for 14 day on polycarbonate membrane</td>
<td>87</td>
</tr>
<tr>
<td>3.7 Percentage of $^{59}\text{Fe}$ found in apical chamber, basal chamber and Caco-2 cells after 1 h incubation</td>
<td>92</td>
</tr>
<tr>
<td>3.8 Effect of calcium on percentage of $^{59}\text{Fe}$ found in apical chamber, basal chamber and Caco-2 cells after 1 h incubation</td>
<td>95</td>
</tr>
<tr>
<td>3.9 Effect of ascorbic acid on percentage of $^{59}\text{Fe}$ found in apical chamber, basal chamber and Caco-2 cells after 1 h incubation</td>
<td>99</td>
</tr>
</tbody>
</table>
3.10 Effect of zinc on percentage of $^{59}$Fe found in apical chamber, basal chamber and Caco-2 cells after 1 h incubation

3.11 Effect of tannic acid on percentage of $^{59}$Fe found in apical chamber, basal chamber and Caco-2 cells after 1 h incubation

3.12 Effect of caffeine on percentage of $^{59}$Fe found in apical chamber, basal chamber and Caco-2 cells after 1 h incubation

4.1 Iron and calcium contents of experimental diets

4.2 Body weight of rats by experimental groups and days of experiment

4.3 Feed intake of rats by experimental groups and weeks of experiment

4.4 Haematological variables in rats with iron-deficient-induced anaemia and subsequently fed different type of diets

4.5 Haemoglobin regeneration efficiency (HRE) values in rats with iron-deficient-induced anaemia and subsequently fed different type of diets

4.6 Serum iron and TIBC values in rats with iron-deficient-induced anaemia and subsequently fed different type of diets

4.7 Transferrin and transferrin saturation values in rats with iron-deficient-induced anaemia and subsequently fed different type of diets

4.8 Weight and iron content of liver in rats with iron-deficient-induced anaemia and subsequently fed different type of diets

4.9 Weight and iron content of spleen in rats with iron-deficient-induced anaemia and subsequently fed different type of diets

5.1 Iron and calcium contents of experimental diets
5.2 Distribution of unabsorbed $^{59}$Fe (% of dose) in the lumen of the gastrointestinal tract of iron deficient mice fed FeSO$_4$ or spirulina diet with or without addition of Calcium carbonate

5.3 Distribution of unabsorbed $^{59}$Fe (% of dose) in the lumen of the gastrointestinal tract of iron normal mice fed FeSO$_4$ or spirulina diet with or without addition of Calcium carbonate

5.4 Distribution of absorbed $^{59}$Fe (% of dose) in various organ of iron deficient mice fed FeSO$_4$ or spirulina diet with or without addition of Calcium carbonate

5.5 Distribution of absorbed $^{59}$Fe (% of dose) in various organ of iron deficient mice fed FeSO$_4$ or spirulina diet with or without addition of Calcium carbonate
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>Estimated population affected by anaemia and iron deficiency, by WHO region</td>
<td>1</td>
</tr>
<tr>
<td>2.1</td>
<td>Iron distribution (mg) and metabolism within the body</td>
<td>8</td>
</tr>
<tr>
<td>2.2</td>
<td>Mechanism of iron absorption</td>
<td>26</td>
</tr>
<tr>
<td>2.3</td>
<td>The influence of iron stores in cells on iron uptake</td>
<td>27</td>
</tr>
<tr>
<td>2.4</td>
<td>Diagram of <em>in vitro</em> digestion/Caco-2 cell culture model utilising radiolabeled iron as developed by Glahn <em>et al.</em>, (1996)</td>
<td>40</td>
</tr>
<tr>
<td>2.5</td>
<td>Life cycle of Spirulina</td>
<td>52</td>
</tr>
<tr>
<td>3.1</td>
<td>Experimental design for iron displacement from spirulina</td>
<td>73</td>
</tr>
<tr>
<td>3.2</td>
<td>TEER value of Caco-2 cells grown on polycarbonate membrane</td>
<td>81</td>
</tr>
<tr>
<td>3.3</td>
<td>Percentage of phenol red diffusion of Caco-2 cell lines grown on polycarbonate membrane</td>
<td>82</td>
</tr>
<tr>
<td>3.4</td>
<td>Ultrastructural features of Caco-2 cell monolayers grown on polycarbonate membranes</td>
<td>84</td>
</tr>
<tr>
<td>3.5</td>
<td>High magnification of brush border showing microvilli and tight junction for cell at 3 days of culture and 13 days of culture</td>
<td>85</td>
</tr>
<tr>
<td>3.6</td>
<td>Protein dye binding response pattern for standard using bovine serum albumin</td>
<td>86</td>
</tr>
<tr>
<td>3.7</td>
<td>Percentage of $^{59}$Fe displaced from spirulina and found in the supernatant after centrifugation (4 000 rpm X 10 min) at different period of time</td>
<td>88</td>
</tr>
<tr>
<td>3.8</td>
<td>Percentage of $^{59}$Fe retained in the cells after centrifugation (4 000 rpm X 10 min) at different period of time</td>
<td>89</td>
</tr>
<tr>
<td>3.9</td>
<td>Total soluble iron present at the end of dialysis and centrifugation</td>
<td>91</td>
</tr>
<tr>
<td>3.10</td>
<td>Total iron uptake by Caco-2 cell</td>
<td>93</td>
</tr>
</tbody>
</table>
3.11 Percentage of soluble iron present after centrifugation at different iron to calcium molar ratios

3.12 Total iron uptake by Caco-2 cell at different iron to calcium molar ratios

3.13 Percentage of soluble iron present after centrifugation at different iron to ascorbic acid molar ratios

3.14 Total iron uptake by Caco-2 cell at different iron to ascorbic acid molar ratios

3.15 Percentage of soluble iron present after centrifugation at different iron to zinc molar ratios

3.16 Total iron uptake by Caco-2 cell at different iron to zinc molar ratios

3.17 Percentage of soluble iron present after centrifugation at different iron to tannic acid molar ratios

3.18 Total iron uptake by Caco-2 cell at different iron to tannic acid molar ratios

3.19 Percentage of soluble iron present after centrifugation at different iron to caffeine molar ratios

3.20 Total iron uptake by Caco-2 cell at different iron to caffeine molar ratios

4.1 Experimental design

4.2 Percentage of haematological parameters changes in iron deficient rats fed different type of diets

5.1 Experimental design

5.2 Body weight of iron deficient mice for a period of 3 weeks

5.3 Body weight of iron normal mice for a period of 3 weeks

5.4 Haemoglobin level of iron deficient mice administered various iron supplement (ferrous sulphate or spirulina

5.5 Haemoglobin level of iron normal mice administered various iron supplement (ferrous sulphate or Spirulina)

5.6 Standard curve of of cpm and µCi unit
5.7 Percentage of administered doses measured at different time period in mice provided different iron supplement
Iron deficiency is the world's most widespread nutritional disorder, affecting both industrialised and developing countries. Iron deficiency and anaemia affect all age groups, particularly the young children and pregnant women and their impact present a major hurdle to national development. The World Health Organization estimated nearly 2 billion people worldwide are anaemic and over twice that number are iron deficient (WHO, 2000). Globally, 39% of preschool children and 52% of pregnant women are anaemic, of whom more than 90% live in developing countries (Figure 1.1).

**Figure 1.1**: Estimated percentage of population affected by iron deficiency anaemia, by WHO region. Source: WHO (2000)