Iron bioavailability for piglets:
The effects of iron status, vitamin C and cooked or uncooked meat

A thesis presented in partial fulfilment of the requirements for the
Degree of Master of Science (Nutritional Science)

at Massey University, Palmerston North, New Zealand

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Abstract

Worldwide, iron deficiency affects more than 1 billion people. People with iron deficiency have symptoms of fatigue, intolerance to the cold and poor behaviour and psychomotor development problems. This is partly because the amount of iron present in food is not the amount that is available to the body.

The bioavailability of iron is a key component in understanding the complexities of iron deficiency.

Using an animal model involving 4-week old anaemic piglets, this study investigated several aspects of iron bioavailability. These were:

The relationship between iron status and iron absorption, the difference in bioavailability of meat iron and non-haem iron and whether supplementary vitamin C can aid in the absorption of dietary iron, and the effect of temperature and cooking of meat on iron bioavailability.

Dietary iron bioavailability was measured both in iron deficient and non-iron deficient piglets, by measuring changes to the composition of the red cell mass, serum iron concentrations and the binding capacity of iron transport proteins over a period of 28-days.

Experiment 1 showed that meat iron was more bioavailable than the inorganic iron in a vegetable based diet. Also, in the anaemic piglet, 500 ppm of vitamin C in the diet was able to enhance the availability of the non-haem iron from a diet consisting of food choices from a typical human diet.

Experiment 2 showed that a diet containing meat iron was able to return iron deficient piglets to haematologic normality more readily than a diet consisting of milk protein and inorganic iron. Also, cooking meat in a steam-heated circulating water bath was beneficial in increasing the digestibility of the diet and also increasing the availability of the meat iron.

The findings of this study reflect the conclusions drawn from similar human studies, thereby providing further evidence of the suitability of the piglet as a model for the human in future studies of iron bioavailability.

Acknowledgements

Sincere appreciation is expressed to my supervisors, Dr. P. C. H. Morel and Associate Professor R. W. Purchas for their guidance and encouragement throughout the study.

I would also like to thank
Mrs. Rosalind Power in analysing blood samples.
Dr. Philip Pearce for laboratory analysis of serum and UIBC iron.
Dr. David Simcock for laboratory analysis of the iron content of feed samples.
Also Ms. Karin Weidgraaf, Ms. Jo Melai, Ms. Laurence De Coster and Mr. Edward James in helping to care for the animals used in this study and also in the collection of blood samples.

The Animal Ethics Committee at Massey University, Palmerston North, approved the experimental protocols of the studies described herein.
Application Numbers: MU Ethics (01/25) and (01/82)

This study was Funded by Meat New Zealand

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Chapter 1

1. Introduction

Iron is an essential element, in the diets of humans and animals. The mineral is widely available in a number of foods, including: meat, liver, shellfish, egg yolk, beans, legumes, dried fruits, nuts and cereals, but despite its availability, iron deficiency is prevalent (Pippard 1995). Worldwide the WHO (World Health Organisation) has estimated that more than 1 billion people have symptoms of fatigue, intolerance to the cold and poor behaviour and psychomotor development problems as a result of iron deficiency. This is partly because the amount of iron present in food is not the amount that is available to the body. To be assimilated, minerals, including iron initially have to be bioavailable. Matzke (1998) proposed that bioavailability of a nutrient can be divided into three constituent parts. These are:

- The nutrient in the intestinal lumen must be available for absorption.
- Absorption and retention of the nutrient in the body.
- Utilization of the nutrient by the body.

The concept of bioavailability of dietary iron is the key to understanding the complexities of iron deficiency. Regrettably bioavailability is not an absolute, and as a result it can be affected by the iron status of the individual, and also the source of the iron. In addition other dietary nutrients can be inhibitory to iron's bioavailability.

The aim of this study was to evaluate the model of the anaemic piglet to assess the bioavailability of iron from the human diet. Prior research has concentrated on the effects of individual nutrients in the diet that may either enhance or inhibit the absorption of iron. Additionally, these studies have used animal models that consumed diets, containing ingredients, which human subjects would not eat at all.

Using 3-week old anaemic piglets, the general objectives of Experiment 1 were:

1. To determine the effect of iron status on iron absorption.

2. To determine the bioavailability of dietary iron from meat and non-meat sources.

3. To determine the effects of vitamin C on the absorption of non-haem iron when fed as part of a complete diet.

Specific objectives were:

- To test the effectiveness of meat and non-meat sources of iron to bring about a return of iron deficient piglets to haematologic normality.

- To compare iron bioavailability in non-iron deficient piglets (21 days old) and iron-deficient piglets receiving non-meat iron daily in their diet.

- To compare iron bioavailability using iron-deficient piglets (21 days old) consuming non-meat iron diets, with either 250 ppm or 500 ppm of supplementary vitamin C, to determine if vitamin C can increase the absorption of non-haem iron.

Food processing affects the initial phase of the bioavailability model, by determining the amount of the mineral that is available for absorption. The meat used in experiment 1 was in a raw, unprocessed state.

The general objective of the second experiment was to determine the effects of cooking temperature on iron bioavailability in meat.

Specific objectives were:

- To determine the effectiveness of iron in meat to return anaemic piglets to haematologic normality.

- To determine the effect of cooking meat on bringing anaemic piglets back to haematologic normality with meat that had been subject to either no heat treatment, heated to 60° C or 90° C.

- To compare iron bioavailability using 2 groups of 5 piglets with either anaemia or positive iron status receiving a non-haem dietary alternative.

Chapter 2

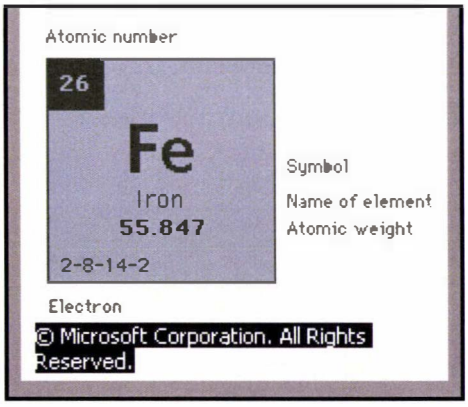
2. Review of Literature.

Kies and Mc Endree (1982) commented that if all nutrients found in food were digested, absorbed and made available to the human or animal, the science and practice of nutrition would be simple. Unfortunately research has shown dietary nutrients differ in their bioavailability (Matzke 1998).

The availability of nutrients is determined by a number of factors, which include the chemical and physical characteristics of the foods containing the nutrients (Lee 1984 and Clydesdale 1982), other constituents of the diet (Hallberg *et al.*, 1987), the digestive and absorptive processes for the specific nutrients (Ferraris and Diamond, 1989) and the physiological condition of the person or animal consuming the food (Roy and Enns, 2000).

This review of literature concentrates on these issues as they relate to dietary iron.

2.1 The physiological role of iron.



Iron is an essential element that is required by humans and animals. It is primarily used in the production of haemoglobin and myoglobin, the oxygen carrying proteins of the blood and muscles, respectively (Totara and Reynolds-Grabowski, 1996). Additionally, elemental iron can exist in two valance states and is therefore able to provide power, through oxidation and reduction reactions, to a number of biochemical pathways (Voet and Voet, 1995). These include those used in the manufacture of enzymes needed for DNA synthesis and ATP (adenosine triphosphate) production. Regrettably the same redox properties of iron can also be detrimental to biological systems generating oxidative radicals, which damage biological components such as lipids, proteins and DNA (Roy and Enns. 2000). Therefore the absorption and metabolism of iron need to be effectively regulated.

2.2 Iron Digestion.

Unable to synthesise elemental iron, the body obtains the iron it requires from the diet, where it is found in a large number of foods including meat, liver, shellfish, egg yolk, beans, legumes, dried fruits, nuts and cereals (Zijp et al. 2000).

There are two forms of dietary iron a) haem iron, which is found mainly in meat and b) non-haem iron that makes up most of the iron in vegetables and cereal grains (Lombard et al. 1997). Haem iron is contained within a porphyrin complex associated with specific proteins. The acid conditions found in the stomach denature the proteins, enabling the porphyrin complex to be released. The porphyrin complex remains unaffected by further changes in pH and is directly assimilated into the enterocyte cells in the wall of the small intestine.

Non-haem iron also relies on mechanical and enzymatic digestion, together with the series of pH changes along the length of the digestive tract, to facilitate its release from the food we eat (Lombard et al. 1997). In addition to freeing iron from other food components, Lombard et al. (1997) proposed digestion also has a further role, in the reduction of iron from the Fe^{3+} (ferric) state to the Fe^{2+} ferrous form. The ferrous form is more readily absorbed across the small intestine, despite the existence of specific transporters for both ferric and ferrous iron (Conrad et al. 1999). The acid conditions in the stomach are thought to initiate the reductive process. Intestinal mucins are then able to chelate the non-haem iron maintaining it in the more soluble ferrous form (Fe^{2+}) (Conrad et al. 1994). Inevitably movement of the chelated iron from the acid environment of the stomach to the small intestine entails an increase in pH. The increased pH can cause the precipitation of chelated iron causing the formation of an insoluble iron residue that cannot be absorbed (Kies and McKendree 1982). The dietary nutrients calcium, phytate and polyphenols inhibit iron absorption by precipitating chelated iron in a similar way (Hallberg *et al.*, 1987).

2.3 Iron absorption mechanisms.

The enterocyte cells differentiate between haem and non-haem iron so that the two sources of dietary iron are absorbed using different pathways (Conrad et al. 1999). Iron is moved from the lumen of the intestine across the apical membrane and into the enterocyte using one of three pathways. Conrad et al. (1999) reported that non-haem iron is absorbed using both the mobilferrin-integrin pathway and a divalent cation transporter (DCT-1), whereas haem iron is absorbed directly into the enterocytes by the haem iron uptake pathway.

DCT-1 is a proton symporter that transports both non-haem ferrous iron and other divalent ions, from the intestinal lumen into the enterocyte. Body iron stores, dietary iron and posttranslational modification regulate the expression of the DCT-1 protein (Conrad et al. 1999).
The mobilferrin-integrin pathway facilitates the absorption of dietary inorganic ferric iron.
The ferric iron is reduced to the ferrous form by a membrane–bound ferrireductase enzyme before entering the absorptive cell via β_3 integrin in combination with mobilferrin, both of which have been identified by Powell et al. (1999) as trans-membrane proteins.

The haem iron uptake pathway facilitates the absorption of haem iron into the enterocyte.
The iron is contained within a porphyrin complex, which enters the absorptive cell as an intact metalloporphyrin. Once inside the cell the porphyrin complex is enzymatically degraded by haem oxygenase, releasing the iron (Raffin et al. 1974).

The basolateral membrane then mediates the transfer of the iron to the blood plasma and then to the rest of the body. As free iron is toxic to biological systems, iron is transported out of the enterocyte and into the body plasma bound to the iron transport protein transferrin (Conrad et al. 1999).

2.4 Iron absorption is related to iron status.

Iron absorption is complicated by regulation of iron uptake occurring at the two interfaces of the enterocyte between the apical and basolateral membrane (Roy and Enns, 2000). The apical membrane of the differentiated enterocyte faces the intestinal lumen and is specialised for transport of haem and ferrous iron into the cell. The basolateral membrane then mediates the transfer of iron transported into the intestinal epithelial cells to the rest of the body (Totara and Reynolds-Grabowski, 1996).
Ferraris (1989) reported that the intestinal absorptive capacity for iron is closely matched to dietary intakes and body requirements, in order to provide just enough absorptive capacity, without wasting biosynthetic energy on unneeded transporters.
The body conserves much of its iron by recycling it from worn out red blood cells, but unfortunately iron is also irreversibly lost from the body in the shedding of hair, epithelial and mucosal cells and in sweat, urine, faeces, bile and blood lost during menstruation. Although these losses are proportionate to body stores of iron (Conrad and Umbreit, 2000), the lost iron needs to be replaced. Also changes in physiological state such as growth and pregnancy also require

increased amounts of iron to be absorbed from the diet for blood and tissue synthesis (Furugouri and Kawabata 1976).

Iron is essential and can only be obtained from the diet; therefore iron transportation proteins need to be continually expressed to operate at low dietary iron levels. But as iron is toxic to biological systems, high levels of iron need to repress the transporter so as to protect the organism against the risk of intoxification.

Therefore when body iron levels fall, iron uptake from the small intestine is increased until the iron reserves are replete again (Roy et al. 2000). Cox and Peters (1980) has shown that during the time the body has a negative iron balance iron absorption is up-regulated, thereby increasing the rate at which the ion transporters become fully saturated with iron, while the rate of transfer in to the enterocyte cell remains unchanged (Ferraris et al. 1989). This is better explained using the Michaelis Menten expression: V_o = Vmax (s) / Km + (s).

Vmax continues to increase until the transporters become saturated with iron and Vmax reaches a plateau, while km remains unchanged.

When high levels of dietary iron are continually consumed, the iron transport protein transferrin becomes saturated with iron; the iron is not absorbed and transported into the blood plasma but excreted as the transporters are repressed to prevent toxicity. Also the rate at which iron is transferred in to the enterocyte cell remains unchanged, therefore any additional iron that may have been transported into the enterocyte cells before they were repressed is trapped, and is subsequently lost from the body as these intestinal cells are sloughed of as the tissue is turned over (Roy and Enns 2000).

2.5 Regulation of iron absorption.

The mechanism by which iron homeostasis is maintained within the body is poorly understood (Roy et al. 2000). Like many of the body's regulatory processes, it is hypothesised that regulation takes place on many different levels, so that when iron levels drop below a critical level iron uptake is increased until the reserves are replenished. Roy at al. (2000) proposed that an imbalance between the rate of erythropoiesis of the marrow and its iron supply induced iron absorption. Furugouri et al. (1976) demonstrated that iron was actively absorbed in the pig during growth. The rapid increase in body mass caused the Fe content in mucosal cells to drop thereby stimulating active absorption of iron.

Iron is distributed throughout the body; typically in humans 60-70% is found in haemoglobin, 20-30 % in the iron storage proteins ferritin and hemosiderin and 3-5 % in myoglobin. Very small amounts are found in haem-containing enzymes and cytochrome (0.2 %) and approximately

0.1% of the body's iron is found in transferrin, which transports iron in the plasma and extra vascular fluid (Worwood 1997).

2.6 Vitamin C increases non-haem iron absorption.

Several studies have shown that ascorbic acid or vitamin C enhances the absorption of dietary non-haem iron. Clydesdale (1982) proposed that vitamin C might aid in the absorption of non-haem iron in several ways as outlined in Table 1.

Table 1: Proposed mechanisms that could aid the absorption of non-haem iron by vitamin C. (Adapted from Clydesdale (1982) in Nutritional bioavailability of iron. Pg 55).

Characteristic	Possible mechanism.
1. pH	The majority of dietary non-haem iron is ferric iron (Fe^{3+}). Prior to assimilation the ferric iron is reduced by a series of pH changes along the digestive tract to the more soluble ferrous form. The acidic properties of vitamin C could contribute to this process both by increasing ionisation and by favouring the ferrous state, which has a greater solubility at the pH of the intestine (10^{-1} M) than does ferric iron (10^{-18} M).
2. Complexation and the oxidation-reduction potential	Nojeim and Clydesdale (1981) found that low stomach pH together with reduction potential differences between ascorbic acid and ferric iron facilitated the formation of a reversible ascorbate-iron complex. This would predominately leave more of the soluble ferrous iron available for absorption. Unfortunately the effectiveness of vitamin C is limited as the continued complex formations cause the pH to increase, changing the ionisation state of the iron. The reversible complex then disassociates leaving more of the ferric form.

In addition to increasing non-haem iron bioavailability and absorption, vitamin C is also required in the transfer of iron from the storage proteins ferritin, hemosiderin and siderophylin to protoporphyrin for haemoglobin synthesis (Monsen 1982).

Table 2: A summary of several studies that evaluated the effectiveness of vitamin C in increasing the bioavailability and absorption of non-haem iron from the diet.

Amount of vitamin C added to diet	Iron source	Effect on Iron bioavailability	Species	Reference
25-1000 mg	$FeCl_3$	2-10 greater absorption	Human	Cook and Monsen (1977)
51-247 mg/ d	$FeCl_3$	No difference	Human	Cook & Reddy (2001)
25 mg 100g/bodyweight		19, 22 and 20% increase in rbc[1], hgb and hct values respectively	Guinea pig	Milne and Omaye (1980)
0.01M	$FeCl_2$	11.5-19.6% increase Increase	Rat	Conrad and Schade (1968)
10.4 g /Kg diet	$FeSO_4$		Rat	Wienk et al. (1997)
330,660,990 (ppm)	$FeSO_4$	3% increase	Pig	Yen and Pond (1981)

[1]rbc: red blood cell; hgb: haemoglobin; hct: haematocrit

Table 2 shows a summary of several studies using human and animal subjects that have evaluated the effectiveness of vitamin C in increasing bioavailability and absorption of non-haem iron from the diet, however it is difficult to compare the results of all these studies as they used different methodologies.

Yen et al. (1981), Milne et al. (1980) and Wienk et al. (1997) used iron-deficient subjects to study the effectiveness of vitamin C in increasing bioavailability and absorption of non-haem iron. This was in contrast to Cook and Reddy (2001) whose subjects were not deficient in iron.

Additionally diet composition varied between the studies. Cook et al. (1997) used a semi synthetic diet containing corn oil, ovalbumin, and dextrimaltose. Whereas Yen et al. (1981), Milne et al. (1980) and Wienk et al. (1997) evaluated the effects of vitamin C using experimental diets formulated specifically for the age and species of the subject, and Cook and Reddy (2001) used a complete 'western-type' diet in their evaluation.

Also the studies used subjects both capable of vitamin C synthesis; (the piglet and rat studied by Yen et al. 1981, and Wienk et al. 1997, respectively) and those for which vitamin C is an essential dietary nutrient. (The human and guinea pig studied by Cook et al. 1997, Cook and Reddy 2001 and Milne et al. 1980, respectively).

The levels and sources of vitamin C used also varied between the studies. The animal studies using synthetic L-ascorbic acid whereas the human study of Cook and Reddy (2001) calculated vitamin C content of the complete trial diet from dietary components.

The measurements of the effectiveness of the vitamin C also varied. The animal studies were repletion type studies that measured the rate at which some blood parameters increased to normal physiological levels. Whereas the study by Cook and Reddy (2001) used extrinsically radio iron–labelled bread and hamburger buns to measure non-haem iron absorption.

Consequently the results of the studies also varied. Cook et al. (1977) gave 63 male subjects a semi synthetic meal containing corn oil, ovalbumin, and dextrimaltose. Vitamin C was added to the meals in varying amounts ranging from 25-1000 mgs. As a result of the incremental increases in the vitamin C content of the diet, a simultaneous increase in the absorption of non-haem iron was also observed. Cook et al reported that the rate of absorption appeared to be logarithmically related to the vitamin C content of the diet so that the larger amounts of vitamin C produced a progressive rise in the increase of iron absorption.

Yen et al. (1981), and Wienk et al. (1997) reported initial increases in iron absorption during the initial stages of their respective studies, but both failed to firmly establish the existence of any linear relationship between the level of vitamin C in the diet and the absorption of non-haem iron in both the piglet and rat; interestingly both of these species are able to synthesise enough vitamin C from glucose to fulfil their requirements. This could suggest that it is solely the iron deficiency that is stimulating the active absorption of iron from the small intestine or alternatively, if vitamin C is involved, its level in the plasma rather than that of the gastrointestinal tract may be an important factor.

However, Milne et al. (1980) was able to reproduce the benefits of supplementary vitamin C on the absorption of non-haem iron. Using 36 male guinea pigs, Milne et al.(1980) evaluated the effect of vitamin C on copper and iron metabolism. The semi-purified diets fed to the animals contained 0, 0.5 or 25-mg/100g vitamin C. Prior to beginning the study the guinea pigs received a daily sub-maintenance dose of vitamin C inducing hypovitaminosis. Milne et al. (1980) then concluded that both plasma vitamin C and iron levels appeared to be related directly to vitamin C intake. Plasma iron was almost twice as high in the animals receiving 25 mg of vitamin C per 100 g body weight than in animals receiving no supplemental vitamin C.

In trying to reproduce desirable experimental conditions to examine the effects of vitamin C on iron metabolism Milne et al. (1980) may have inadvertently created a situation where vitamin C appeared to be related to the absorption of non-haem iron when in fact the guinea pig's body was replenishing the small body stores of vitamin C.

Cook and Reddy (2001) compared the effect of vitamin C on non-haem iron absorption from a complete diet rather than from a single meal and found that the facilitating effect of vitamin C on iron absorption from a complete meal was far less pronounced than that from single meals. However in this study Cook and Reddy used both male and female subjects who had a positive iron status (serum ferritin concentrations that were greater than 12 µg / L). The regulative

mechanisms proposed by Roy et al. (2000) suggest that the radio-labelled iron would not be actively absorbed in these circumstances and attempts to enhance iron absorption in a subject with positive iron status would also be ineffective; as the toxicity of excess iron would initiate the repression of the iron transporters in the small intestine until such time when body iron levels began to decrease. Also the role of Vitamin C in enhancing the absorption of iron is in maintaining it in the Fe^{2+} state rather than increasing the rate at which the body assimilates it and to do this the vitamin C has to be present in the stomach at the same time as the non-haem iron (Cook et al 1977), which could not be guaranteed when subjects were allowed to consume a self selected diet.

Therefore the evidence from these studies suggests that iron status and diet composition also contribute to the effectiveness with which vitamin C increases the bioavailability and absorption of non-haem iron from the diet.

2.7 Effect of meat on iron bioavailability

In addition to vitamin C, the principal enhancer of non-haem iron absorption in the diet is meat (Seth and Mahoney 2000). During digestion, stomach acids denature the meat proteins prior to them being broken down into small peptides and free amino acids (Totara 1996). Kroe et al. (1963) found that nine amino acids found in meat were able to increase the uptake of ferrous iron, including histidine, glutamine, glutamic acid and methionine and just three were able to increase the uptake of ferric iron. These were cysteine, histidine and lysine. Cysteine like vitamin C is a reducing agent that assists in the conversion of ferric iron into its ferrous form. Unlike cysteine, histidine is not a reducing agent (Stryer 1995); therefore the increased uptake of iron must be because of a factor other than reduction.

Kies and McKendree (1982) proposed several ways that histidine could contribute to the increased bioavailability of haem iron. These are shown in Table 3.

Table 3: Proposed mechanisms that could explain why histidine, cysteine and lysine can increase the bioavailability of haem iron (Adapted from Kies and McKendree (1982) in Nutritional bioavailability of iron Pg 185).

Characteristic	Proposed mechanism of histidine
1. Buffering changes in pH	Amino acids like iron are sensitive to changes in pH, which cause changes to their ionisation state. Therefore the amino acids could act as buffering agents in the intestine and delay the increase of the pH towards neutrality where the oxidation of iron forms insoluble precipitates.
2. Chelation	Alternatively amino acids can form iron amine chelates that act to enhance iron absorption.
3. Transportation	Amino acids can stimulate iron transport systems within the animal thereby increasing the absorption of iron.

2.8 The effect of heat processing of meat on iron bioavailability.

The digestibility and nutritive value of protein is affected by heat processing (Jansuittivechakul et al. 1985). This is because proteins are sensitive to both pH and heat, which cause them to denature (Stryer 1984). Meat contains both haem and non-haem iron (Lombard et al. 1997) Studies by Carpenter and Clark (1995) and Kristensen and Purslow (2001) have found that during heat processing the haem iron content of meat and meat products decreases. The decrease in the haem iron content of the meat is accompanied by an increase in the non-haem iron content. Table 4 shows a summary of several studies that have evaluated the effects of heat processing on the haem and non-haem iron contents of meat and meat products.

Table 4: A summary of the effects of heat processing on the haem and non-haem iron content of meat and meat products.

Meat or meat product	Processing	Effect on Iron bioavailability	Species	Reference
Ground beef	Raw, boiled autoclaved	No effect	Rat	Jansuittivechakul et al. (1985).
Ground Beef	Baking, Microwave	Decrease	In-vitro	Schricker and Miller (1983)
Beef (baked)	Baked	Decrease	Rat	Rotruck et al. (1979)
Pork	Steam heated water bath	Decrease	In-vitro	Kristensen and Purslow (2001)
Rabbit and Beef precipitates	Direct heat	Decrease	In-vitro, Human	Garcia et al. (1996)

The similarities in methodology of the three *in-vitro* studies enable comparison of results to be made.

Kristensen and Purslow (2001) used the *longissimus* muscles taken from pig carcases 24 hrs after slaughter to examine the effects of heat processing on the haem to non-haem iron ratio in meat. Meat was trimmed of visible fat and connective tissue and coarsely minced through a 3.7-x1.6 cm plate. Heat treatment of the meat was performed in thermostatically circulating water baths for 2 hrs followed by cooling on ice water. Kristensen and Purslow found that as temperature was incrementally increased above 55ºC the haem iron content of the meat gradually decreased. This was followed by an increase in the non-haem iron content of the meat, which increased when heat-processing temperature exceeded 80°C. As little or no loss in haem iron content occurred in samples heat-treated to temperatures below 55ºC it can be concluded that the myoglobin containing the iron was still intact. Also the non-haem iron content is stable until temperatures are in excess of 80°C.

This is consistent with the findings of Schricker and Miller (1983) who used several experiments to determine the effects of cooking and chemical treatment on haem and non-haem iron in meat. Small 5 g samples of ground beef were subjected to the effects of baking (176ºC) for 20 –40 minutes and microwave cooking for 0.5 and 3 minutes (600 watts). Schricker and Miller showed the existence of a linear increase in the non-haem iron content of the meat and the time of exposure to the heat treatment. The experiment was later refined and repeated with much larger samples of ground beef (225-270 g). Taking both surface and interior samples of the cooked meat for iron evaluation. The results showed that the smaller samples subject to harsh cooking

treatment had the greatest decrease in haem iron, and that the haem iron content of the meat surface deteriorated more than the interior.

The method of Garcia et al. (1996) was a little less orthodox. Samples of rabbit and beef meats were subject to direct heat treatment until the degradation of the meats metmyoglobin had caused the colour of the meat to change from red to brown. The visual interpretation of the colour change may have introduced error into the experiment (especially as there are differences in the colour of meat from different muscle groups, and also the surface colour of the meat changes when exposed to oxygen.) rather than subjecting samples of meat to a specific temperature treatment for a given period of time. However, the decrease in the haem iron content of the meat as a result of heat processing was consistent with other studies.

Unfortunately comparisons of in-vivo studies between different species are more difficult Jansuittivechakul et al. (1985) and Rotruck and Luhrsen (1979) both used the rat to model iron bioavailability and absorption in repletion type studies. They found that in anaemic rats consuming experimental diets, that availability of inorganic iron (ferrous sulphate) from the control groups was higher than either meat (cooked beef) or haemoglobin iron. The opposite occurs in the human. This suggests that the rat may not be a good model in which to study iron bioavailability and absorption.

2.9 Measures of iron status.

As body iron is found in the blood, iron status can be effectively determined by measures of the number and percentage of red cells, blood haemoglobin concentration, the mean corpuscular volume, and mean corpuscular haemoglobin concentration.

The accepted values for these haematologic parameters in the pig are shown in Table 5.

Table 5: Accepted physiological values of red blood cell parameters in the healthy pig. (Egeli et al. 1998).

Blood Parameter	Accepted Range
Red blood cell number per unit volume of blood.	5-8 x 10^{12} cells / L
Blood haemoglobin concentration in whole blood.	110-170 g / L
Haematocrit (RBC volume/whole blood volume).	0.37-0.50 L / L (or 37-50 %)
Mean red blood cell volume.	50-68 f L
Mean red blood cell haemoglobin.	14.4-20.1 pg (weight per red blood cell).
Mean corpuscular haemoglobin concentration within the red blood cell	300-340 g / L

Red blood cells are manufactured in the bone marrow. They are distinct from other cells in that they lack a nucleus and other cellular organelles necessary to reproduce and carry out extensive metabolic activities. They have a natural lifespan of approximately 120 days. The number of red cells in the circulatory system is homeostatically regulated, so that the number of cells entering the circulatory system equals those removed and destroyed, although an exception to this occurs during the physiological states of growth and pregnancy when the number of red cells entering the circulation increases due to increases in blood volume. The number of red cells or alternatively the percentage of red cells in circulation (haematocrit) can be used to identify iron deficiency (Worwood 1997).

The purpose of the red blood cell is to deliver oxygen to the body's cells and remove carbon dioxide. They are able to accomplish this, as they contain the specialised protein haemoglobin. "The haemoglobin molecule contains four peptide chains that are held together by non-covalent attraction. Each of the polypeptides contains a tightly bound haem, a substituted porphyrin with a central iron atom. The four chains are packed together and the haem groups are located in crevices near the exterior of the spherical molecule, one in each of the four subunits. Allosteric interactions enable the haemoglobin to co-ordinately transport oxygen, carbon dioxide and H^+ ions" (Stryer 1984). Measuring blood haemoglobin concentrations can also identify iron deficiency (Worwood 1997). Additionally measurements of blood haemoglobin concentrations in repletion type studies can be used to identify the amount and type of dietary iron that can restore the body's iron balance most effectively.

A differential white blood cell count is used to monitor the health status of subjects. A high white blood cell count is usually indicative of infection (Totara 1996) and can result in a depression in the levels of serum iron (Beisel et al. 1974). While specific increases in the type of white cell can be used to point to the infectious agent involved.

Table 6 shows the accepted ranges of the white blood cell parameters in the pig.

Table 6: Accepted physiological values of white cell parameters in the normal healthy pig. (Egeli et al. 1998).

Blood parameter	Accepted range X 10^9 cells / L	% Of white cell mass
White cell	10-23	100
Cell type		
Neutrophil cells	2.5-10	26.6-56.7
Lymphocyte cells	7.0-15.5	35.5-62.0
Monocyte cells	0.32-2.0	1.6-8.8
Eosinophil cells	0.08-1.76	0.1-5.6
Basophil cells	0-0.3	0-2.7

2.10 Using an animal model to study iron deficiency in the human

Nutrition studies, using human subjects are difficult to carry out. Therefore animal studies are often initially used to evaluate the costs and benefits of dietary nutrients. Several animal species have been considered as a model for nutritional studies in the human. These include the monkey, guinea pig (Narasinga et al. 1977), and rat (Wienk et al. 1997)). More recently the pig has been shown to be a promising model due to the similarities in digestive and absorptive processes (Moughan and Rowan 1989). Using scaling techniques Moughan *et al* (1992) estimated that the digestive tract of a 3-week old piglet could be used in comparative studies to represent a 3-month old infant.

The pig has several characteristics that made it the most appropriate animal model in evaluating iron bioavailability from a complete diet. These are outlined in Table 7.

Table 7: Characteristic of the pig that make it an appropriate model for studies of iron availability, for humans.

Characteristic	
1. Omnivore	Like the human the pig is omnivorous enabling it to digest food from both animal and plant origin (Encarta Encyclopedia 2000).
2. Eats most foods.	As the pig eats most foods that are consumed by humans, the experimental diets can be formulated to contain foods typical of the human diet (Moughan and Rowan 1989).
3. Iron status	After birth the piglet quickly becomes iron deficient, as it is born with limited iron reserves and the milk produced by the sow contains very little iron (English et al 1984). Iron deficiency is usually prevented in the farmed pig by the administration of iron by intramuscular injection soon after birth. Therefore delaying the administration of iron into the piglet provides a similar scenario to that of a child or adult suffering from iron deficiency (Morel 2001 personal communication).
4. Vitamin C synthesis	The pig can synthesise enough vitamin C from glucose to meet its own requirements, from approximately 1 week of age (De Rodas et al. 1998). Lee (1984) reported several studies, which found that the reducing property of organic acids could increase the absorption of non-haem iron. Therefore, vitamin C can be added to the pig's diet allowing its role as a reducing agent in the digestive tract to be

explored, without it being utilised by the animal.

Despite these beneficial characteristics and apparent physiological similarities, care is always needed when the results from an animal study are extrapolated to the human.

2.11 Summary

The physiological roles of iron include, the production of haemoglobin and myoglobin, and in the provision of power to a number of biochemical pathways.
When the body has a positive iron balance iron is stored in small metabolite pools enabling iron to be continually available to be used in its physiological roles.
Iron stores can be depleted either due to increases in requirements for growth and pregnancy or insufficiencies in the amounts of iron being obtained from the diet, the iron balance in the body moving from positive to negative. Restoring the balance of iron in the body is difficult despite the fact that the depleted iron stores assist in iron being absorbed from the gastrointestinal tract. This is because the iron content of food is not that available to the body through absorption. The bioavailability of dietary iron is not an absolute value; iron status, food processing and other components of the diet can influence how much of the dietary iron is absorbed.
Animal models can be a valuable resource, aiding in the quantification of bioavailability. The most suitable animal models have digestive and metabolic processes that are comparable to those of the human. In studies of iron bioavailability the piglet may be more suitable than the rat model, as both the piglet and the human assimilate the two sources of dietary iron in similar ways.

Chapter 3

3. The use of piglets to evaluate iron bioavailability

3.1 Introduction

Worldwide iron deficiency affects more than one billion people. They have symptoms of fatigue, intolerance to the cold and poor behaviour and psychomotor development problems. This is because the amount of iron present in food is not the amount that is available to the body. There are two forms of dietary iron; haem iron mainly found in meat and non-haem iron that makes up most of the iron found in cereal and grains.
The availability of non-haem iron is particularly susceptible to pH changes that occur along the digestive tract. Additionally this type of iron can also form complicated associations with other dietary nutrients, which can change the amount of iron that is available in the small intestine for absorption. Haem iron is less affected by pH; thereby increasing its availability.
The predisposition of non-haem iron to changes in ionisation state identified several nutrients that are able to either buffer changes in pH or maintain non-haem iron in its Fe^{2+} state enabling a greater proportion of iron to be available for absorption. These are some amino acids derivatives found in meat and also organic acids.
The body's capacity to absorb iron is tightly regulated; because excess iron is toxic to biological systems, therefore the absorptive capacity of the small intestine is closely matched to dietary intakes and body requirements. So that in states of iron deficiency within the body, iron is actively absorbed from the gastrointestinal tract.

The objective of this study was to evaluate the effect of iron status on iron absorption, to quantify the biological effectiveness of the different forms of iron in returning an iron deficient subject to haematologic normality using a repletion study and additionally whether the strategic use of vitamin C, an organic acid, is able to increase the bioavailability of non-haem iron in anaemic 3-week old piglets.
A second experiment evaluated the effect of cooking temperature on iron bioavailability from meat.
The data obtained from both experiments is compared with data from human studies with the aim of validating the piglet as a suitable animal model for further bioavailability studies.

3.2 Materials and methods

3.2.1 Animals

Experiment 1

Five sows on a commercial pig farm were randomly selected from a group of twelve, which represented the number of sows that are farrowed each week from a 300-sow commercial pig unit. Each of the hybrid sows had been cross-mated to a sire line boar, producing slaughter generation progeny.

After a gestation period of 115 days, each of the five sows was induced to farrow. The sows produced 19, 14, 13, 14 and 13 piglets, respectively. Using only the male piglets from each litter, 39 piglets were identified by the insertion of a numbered ear tag. Of these piglets, 14 were injected shortly after birth with 200 mg of iron (as iron dextran), while the remaining 25 piglets received no iron injection. The justification in identifying more piglets than were necessary for this study was to account for any variation in weaning weight and also any pre-weaning mortality that may occur.

The piglets receiving no supplementary creep feed during the pre-weaning period. Cross fostering was permitted, occurring in two instances.

The animals were weaned at 21 days. At weaning, the 39 piglets were weighed. Animals used in the experiment were chosen so that each treatment group would contain 5 piglets (n=5), one from each of the litters. (An analysis of variance was used to determine that there was no significant difference between piglet weights). Each group would consist of one piglet that had been injected with iron and 4 others that had not received any iron.

The piglets were transported to Massey University's Intensive Animal Research facility, where they had a two-day period of acclimatisation, prior to the commencement of the study. During this transition period piglets were offered a liquid starter diet consisting of skim milk powder (40%) and casein (10%) at a rate of 400 g per piglet per day. After this brief period of acclimatisation, dietary treatments were randomly assigned to the groups.

Experiment 2

Using animals from the same herd as experiment 1. 5 sows were randomly selected and induced to farrow after a gestation period of 115 days.

Using both male and female piglets, 8 animals were randomly selected from five litters. Each of the 40 piglets was identified by the insertion of a numbered ear tag.

15 of the 40 piglets (3 from each litter) were randomly chosen and were injected shortly after birth with 200mg iron. The remaining 25 piglets (5 from each litter) received 60 mg iron as iron

dextran. As in experiment 1 the justification in identifying more piglets than were necessary for this study was to account for any variation in weaning weight and also any pre-weaning mortality that may occur.

The piglets were reared on the sow and received no supplementary creep feed during the pre-weaning period. Cross fostering was permitted, occurring in two instances, and 2/5 litters were multi-suckled. The piglets were weaned at 21 days.

At weaning, the 40 piglets were weighed. Animals used in the experiment were chosen so that each treatment group would contain 5 piglets (n=5), one from each of the litters. (An analysis of variance was used to determine that there was no significant difference between piglet weights). Each group would consist of one piglet that had been injected with 200mg iron and 4 others that had received 60mg iron.

The piglets were transported to Massey University's Intensive Animal Research facility, where they had a period (7 days) of acclimatisation prior to the commencement of the study. This differs from the initial study where piglets had a 2-day acclimatisation period. The acclimatisation period was extended so that feeding regime would be more established at the beginning of the study, thereby reducing the incidence of feed refusal and low intakes that occurred in the initial experiment. During this transition period piglets were offered a liquid starter diet consisting of skim milk powder and casein at a rate of 400 g per piglet per day. After the period of acclimatisation piglets were randomly allocated to treatment groups using sex as a block.

3.2.2 Housing

Experiment 1

Piglets were individually housed, in metabolism crates. The crates were situated in a controlled environment of Massey University's Intensive Animal Research facility.

The metabolism crates were manufactured from galvanised metal, each crate measuring 1.5 x 0.5m. The crate floor was made from punched metal, allowing urine and faeces to fall to the floor of the room, thereby preventing caprophagy and the ingestion of iron excreted in the faeces. Recycled plastic covered 1/3rd of the floor area and provided a comfortable laying area for each piglet. An infrared heat lamp provided additional heat so that the temperature was maintained within the animals thermo-comfort zone at 29°± 1.5° C.

Each of the metabolism crates contained a removable 400 mm, ad-lib feeder (Stallion plastics New Zealand) and water was available at all times through a bite nipple drinker.

Experiment 2

As with experiment 1, animals were individually housed in metabolism crates that were situated in an environmentally controlled room in Massey University's intensive animal research facility. The pen dimensions and layout of the metabolism crates were as in experiment 1.

3.3 Experimental design

Experiment 1

The experiment was a one-way design with repeat measures, which used 3 experimental treatments and 2 controls, as shown in Table 8.

Table 8: Characteristics of the five treatment groups used in experiment 1.

Group	Fe at birth	Diet	Vit C (ppm)	No animals
C	0 mg	Control	0	5
CV250	0 mg	Control + Vit C	250	5
CV500	0 mg	Control + Vit C	500	5
Meat	0 mg	Meat	0	5
C+	60 mg	Control	0	5

Experiment 2

The experiment was a one-way design with repeat measures, which used 3 experimental treatments and 2 controls, as shown in Table 9.

Table 9: Characteristics of the five treatment groups used in experiment 2.

Group	Fe at birth	Diet	Meat cooking conditions	No animals
C	60 mg	Control	-	5
M60	60 mg	Meat	~60°C for >60 min	5
M90	60 mg	Meat	~90°C for >60 min	5
Mr	60 mg	Meat	Uncooked	5
C+	200 mg	Control	-	5

The control diet was fed to both C and C+ groups who differed only in iron status. The meat diet was fed to three treatment groups and contained a meat fraction that was either raw or had been cooked to about 60° or 90° C.

3.4 Diets and Feed Management

3.4.1 Diet

Experiment 1

Each piglet received one of four diets, as shown in Table 10. The control diet was fed to both the C and C+ groups who differed only in iron status. The diets used mainly food choices from human diets, but were balanced for digestible energy (DE) and Lysine enabling them to be fed to the piglets without any detrimental effects.

The composition of the experimental diets are shown in Table 10

Piglets were fed their respective diets from 23 days of age (experimental day 0) for a 28-day experimental period.

Table 10: The ingredient composition of the experimental diets (%) for experiment 1 on an as-fed basis, including the additional mixing water.

Ingredients %	Group C, C+	Cv250	Cv500	Meat
Peas	10	10	10	0
Skim milk powder	10	10	10	5
Soybean meal	4	4	4	0
Soybean oil	2.5	2.5	2.5	5
Threonine	0.1	0.1	0.1	0
Methionine	0.125	0.125	0.125	0.075
Vitamins + minerals (Excl. Fe)	0.1	0.1	0.1	0.1
Cellulose	1.5	1.5	1.5	1.5
Dicalcium phosphate (DICP)	1	1	1	1.25
Sodium Chloride (NaCl)	0.075	0.075	0.075	0.1
Meat	0	0	0	25
Water	63	63	63	50[1]
Wheat starch	7.5	7.5	7.5	11.96
Fe heptasulfate	0	0	0	0.01[2]
Vit C Rovimix^{R}	0	0.0125	0.0250	0.00075[2]

[1]Less water was added to the meat diet because the meat contained approximately 75% water.
[2]Fe heptasulfate and Vit C were added to the meat diet to mach the level in the C and C+ diets.

Table 11 shows the calculated and analysed nutrient composition of the experimental diets.

Table 11: Calculated and analysed nutrient composition the experimental diets used in Experiment 1 on an as-fed basis.

Content (on an as fed basis)	Control	CV250	CV500	Meat
Calculated				
Digestible Energy DE (MJ /Kg	5.9	5.9	5.9	5.82
Lysine (g/ kg)	5.27	5.27	5.27	5.79
Ca (g/ kg)	4.14	4.14	4.14	4.04
Total H_2O ml/L reconstituted diet	672	672	672	711
Analytical[1]				
Total Fe (ppm)	43.4	40.6	45.9	41.2
% Haem	0.8	0.8	0.9	7.7
% Non-haem	99.2	99.2	99.1	92.3

[1]Dr Roger Purchas personal communication

Experiment 2

The ingredient compositions of the experimental diets for Experiment 2 are shown in Table 12.

Table 12: The ingredient compositions of the experimental diets (%) for Experiment 2 on an as-fed basis including additional mixing water.

Ingredients %	Group C, C+	M60	M90	Mr
Skim milk powder	5	0	0	0
Soybean oil	5	5	5	5
Threonine	0.05	0	0	0
Methionine	0.125	0.05	0.05	0.05
Vitamins + minerals	0.08	0.08	0.08	0.08
Cellulose	1.5	1.5	1.5	1.5
Dicalcium phosphate (DICP)	1.5	1.5	1.5	1.5
Sodium Chloride (NaCl)	0.05	0.05	0.05	0.05
Meat	0	25	25	25
Water	67.5	50*	50*	50*
Wheat starch	11.72	11.72	11.72	11.72
Fe heptasulfate	0.0035	0.00025	0.00025	0.00025
Casein	7.5	5	5	5

* Less water was added to the meat diet because the meat contained approximately 75% water.

Table 13 shows the calculated and analysed nutrient composition of the experimental diets.

Table 13: Calculated and analysed nutrient composition the experimental diets used in Experiment 2 on an as-fed basis.

Content (on an as fed basis)	C, C+	Mr	M60	M90
Calculated				
Digestible Energy DE (MJ /Kg)	6.0	5.91	5.91	5.91
Lysine (g/ kg)	7.7	8.6	8.6	8.6
Ca (g / kg)	5.2	4.6	4.6	4.6
Total H_2O ml/L reconstituted diet	713	716	716	716
Analytical[1]				
Total Fe (ppm)	25.3	26.1	26.7	30.5
% Haem	5.9	31.3	27.6	21.8
% Non-haem	94.1	68.7	72.4	78.2

[1]Dr Roger Purchas personal communication

Piglets were introduced to their respective diets on day 0 of a 28-day experimental period. The meat was prepared from 120 kg frozen topside of beef. This was thawed overnight at room temperature, prior to being minced through an 8 mm plate. The liquid exudate from the thawing meat was collected and added to the minced beef.

After mincing, the beef was divided into 3 x 40 kg batches and packaged into 1 kg polythene enclosed cylindrical packs (diameter of 70 mm). The first 40 x 1 kg of beef was frozen without cooking to –17.5° C. The remaining 80 x 1 kg of beef were cooked in steam-heated water baths, 40 kg at 62° C and 40 kg at 92° C, respectively. The M60 rolls were in the water bath for approximately 2.25 hours and the internal temperature of a sample roll was at > 57° C for more than 60 minutes (maximum temperature = 59.2° C. The M90 rolls were in the water bath for approximately 2 hours and the internal temperature of a sample roll was > 84° C for about 60 minutes (maximum temperature =88° C). The packages were then rapidly cooled and frozen. The individual 1 kg packages of meat were then thawed 24 hrs at 4° C prior to them being incorporated into the meat diets.

3.4.2 Feed management

Experiment 1

Piglets were fed twice daily (at approximately 8.00 am and 5.00 pm). The provision of two smaller meals rather than a single feed would ensure any food that was consumed would be fully digested and released into the small intestines in small quantities. These conditions maximise the absorption of nutrients. The amount of feed offered per piglet began at 200g / feed/day and increased incrementally by 200 g every 4 days. This methodology was determined during a pre-trial review to be more appropriate than calculating feed intake base upon liveweight. The

justification for this was the aim of the study was to determine bioavailability of dietary iron by presenting each piglet with a fixed amount of iron each rather than to achieve maximum rates of growth day.
The liquid diet was freshly prepared for each feed by:

- Weighing the mixing water
- Weighing the dry ingredients
- Combining the dry ingredients with mixing water in a food processor and blending the water and dry ingredients together for 15 seconds to produce a liquid diet.
- In the case of the meat diet, the minced meat (100% visual-lean bull beef) was added to the mixing water prior to the addition of dry ingredients.

A raspberry liquid concentrate flavour (manufactured by Hansell New Zealand) was added at a rate of 1g /100g dry diet to increase the palatability of the diet because of poor intake during the acclimatisation period.
Pigs had unlimited access to the allocated feed from one feed to the next. Prior to each feeding the feeder was removed from the metabolism crate, the weight of feed refusals was recorded, and any uneaten food was removed and discarded. The feeders were then washed and replaced.

Experiment 2

As in experiment 1, the piglets in experiment 2 received a fixed amount of food each day that increased incrementally every fourth day. The diets were prepared using the method detailed in experiment 1 by being combined with an equal volume of water to provide a liquid diet. The diets used in experiment 2 did not contain raspberry flavouring because piglets had an extended period of acclimatisation during which feed intake was good.

3.5 Blood Samples

Experiments 1 and 2

A blood sample was taken on days 0, 6, 14, 21 and 27 from each of the piglets in experiments 1 and days 0, 7, 14, 21, and 28 in experiment 2 under halothane anaesthesia.
Approximately 8 ml of blood was drawn from the jugular vein of each animal using either a 20G: 1" or 1.5" needle and collected in 5 ml and 3 ml vacutainer tubes (Manufactured by Becton Dickinson Vacutainer systems, Europe). The 5 ml tubes, which contained no added anticoagulant were stored overnight at 4º C. The clots of blood were then unstuck from the sides of the vacutainer and the vacutainer centrifuged at 4º C, at 3000 rpm (g value 3040) for 20 minutes

until the serum had separated from the other blood products. The serum was poured into pre-labelled duplicate vials and frozen at –20º C.

The 3 ml vacutainer contained the anticoagulant EDTA (0.068 ml of 7.5% (K_3) EDTA solution (5.1 mg)) that prevented clotting of the blood sample. These samples were not stored but sent to the Institute of Veterinary, Animal and Biomedical Science (Massey University) immediately for analysis using an "Advia 120" electronic cell counting apparatus (manufactured by the Bayer Corp. Tarrytown, N. York).

The "Advia 120" analysed the blood samples measuring the parameters shown in Table 14. The accepted physiological values of the red blood cell parameters in a normal healthy pig are shown in Table 5.

Table 14: Red blood cell parameters, units and abbreviations.

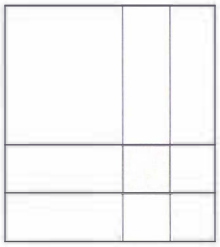
Blood parameter	Abbrev.	Unit	Measurement
Red blood cells	RBC	10^{12} cells/ L	Direct
Blood haemoglobin concentration	HGB	g / L	Direct
Haematocrit	HCT	L / L	Direct
Mean Cell Volume	MCV	fL	
Mean cell haemoglobin	MCH	p g	Calculated as: HGB/RBC
Mean Corpuscular, haem concentration	MCHC	g / L	Calculated as: HGB/RBC x MCV
Corpuscular haemoglobin constant	CHCM	g / L	Calculated as: MCHC or MCH/ MCV

The same apparatus classified red cell characteristics by red cell volume (V) and haemoglobin concentration (HC) using a 3 x 3 matrix.

Red cells are arranged in categories that have cell dimensions between V<30 fL, HC<280 g/ L and V>80 fL, HC >410 g / L, using the labelling M1 through to M9. The layout of the matrix is shown in Table 15. The alignment of the matrix gridlines is dependent on species, sex and age. The alignment of the matrix for the piglets used in this study is shown adjacent to Table 15. This places red blood cells of optimum size in the M5 category (Egeli et al. 1998).

Table 15: Red cell classification using a 3 x 3 matrix

RBC V/HC Gridlines



Red blood cell matrix		Haemoglobin concentration		
		HC <280	HC= 280-410	HC >410
cell volume	V>80	M7	M8	M9
	V=30-80	M4	M5	M6
	V<30	M1	M2	M3

The electronic cell counter was also able to provide a differential white cell count, isolating the number of type of white cells in each of the blood samples as shown in Table 16. The accepted physiological values of the white blood cell parameters in a normal healthy pig are shown in Table 6.

Table 16: White blood cell parameters, units and abbreviations.

Blood parameter	Abbreviation	Unit
White blood cell	WBC	10^9 cells / L
Neutrophil cell	NEUT	10^9 cells / L
Lymphocyte cell	LYMPH	10^9 cells / L
Monocyte cell	MONO	10^9 cells / L
Eosinophil cell	EOS	10^9 cells / L
Basophil cells	BASO	10^9 cells / L

Serum iron and TIBC and UIBC

Total serum iron and unsaturated iron binding capacity were measured using Roche Fe diagnostic kits in a "Cobas Fara IV" analyser by the Institute of Food Nutrition and Human Health (Massey University). Serum iron was determined at acid pH in the presence of a reducing agent ferrozine. Under these conditions serum proteins are precipitated and iron is released from transferrin. Spectrophotometric analysis (560 nm) was then used to determine the serum total iron concentration.

Serum unsaturated iron binding capacity (UIBC). At alkaline pH a standard amount of ferrous iron is added to serum and is sequestered by transferrin, filling all available binding sites on the protein. The remaining unbound ferrous ions are then measured calorimetrically by use of ferrozine. The difference between the amount of unbound iron and the total amount of iron

added is the UIBC. Serum total iron binding capacity (TIBC) is the sum of serum total iron and UIBC.

3.6 Data analysis

An analysis of variance (ANOVA) was used to compare the mean initial weight of the five groups of piglets.

Statistical analysis was carried out using a general linear model, within the Proc GLM procedure of the SAS System, 8.1. (SAS institute Inc. Cary, NC, USA)

In Experiment 1 physical production, red blood cell parameters and an evaluation of the white cell counts were carried out using the statistical model:

$$y = \mu + group_i + pig_j(group) + week_k + week \times group + err_{ijk,}$$

Where:

y = variable.

μ = overall mean.

Group= fixed effect of $_i^{th}$ group (see Table 8).

Pig= random effect $_j^{th}$ pig in the $_i^{th}$ group.

Week= fixed effect of the $_k^{th}$ week.

Week x group = interaction between week and group.

Err = refers to the residual error.

In Experiment 2 the statistical model was expanded to account for sex differences in the piglets:

$$y = \mu + group_i + sex_j + sex \times group + pig_k(sex \times group) + week_l + week \times group + err_{ijkl.}$$

Where:

μ = refers to the overall mean.

Group = fixed effect of $_i^{th}$ group see Table 8.

Sex = fixed effect gender of the piglet.

Sex x group = interaction between sex and group

Pig = random effect $_k^{th}$ pig in the $_i^{th}$ group.

Week= fixed effect of the $_l^{th}$ week.

Week x group = interaction between week and group.

Err= refers to the residual error within the model

Comparisons between the treatments in both experiments were made using least significant differences (LSD).

The retention of body haemoglobin iron was estimated by:
Initially using the following equation to calculate body haemoglobin iron content as:

Body haemoglobin iron = Liveweight x 0.07 x haemoglobin concentration x 0.00335
(g) (g) (g/L)

Where:
0.07 = weight of blood in a piglet as a proportion of body weight (7%).
0.00335 = Fe weight as a proportion of haemoglobin weight.
(value from Thoren-Tolling, 1975)

Then using regression analysis between cumulative iron intake (FEI) and whole body haemoglobin iron (HGB Fe) so that the equation for iron retention in each piglet becomes:

$HGB\ Fe_i = a + b \times FEI_i + e_i$

Where

HGB Fe = Body haemoglobin iron retention
a= intercept (the expected value of the dependant variable when the x-variable is zero).
b= slope (the expected change in the dependant variable given a unit change in the x-variable.
FEI= cumulative iron intake (feed intake x total iron content of the diet).
e = residual error

Then the individual intercept (a) and the slope (b) were analysed with the following linear model:

$Y_{ij} = \mu + group_i + e_{ij}$

Where:

Y= variable
μ = overall mean
Group = fixed effect of the $_i^{th}$ group.
e = residual error

Comparisons between the groups were made using least significant differences (LSD).

Initial haematology results showed unequal CHCM and MCHC values indicating that the settings used by the "Advia 120' in cell counting, laser light scattering, and flow cytometry were inappropriate for the severity of iron deficiency. A consultant from the machine's manufacturer (Bayer) accounted for these differences by taking the data generated during the haematology analysis and adjusting it to account for low body Fe. The values reported for red blood cells, haematocrit and MCHC in both experiments 1 and 2 were calculated using these adjusted settings.

Chapter 4

4. Results (experiment 1).

Table 17 the shows the significance of the effects of group, pigs within group, week and week x group on feed intake and growth rate. The statistical model explained 94% (r-squared = 0.94) and 90% (r-squared = 0.90) of the variation between the groups for intake and growth rate respectively over the 4-week study period.

Table 17: Significance levels of the effects of group, pigs within group, week and week x group on feed intake and growth rate.

	Group	Pig (group)	Week	Week x group	R^2
Intake	***	***	***	***	0.94
Growth rate	***	***	***	***	0.90

ns: not significant p>0.05; * p<0.05; ** p< 0.01; *** p<0.001

4.1 Intake

Statistically significant differences (P< 0.001) were observed between the groups and there were also significant differences between weeks, and pigs within groups. There was also a significant interaction between week x group. The variable week x group was tested to identify changes that took place over the 4-week study period. The results presented in Table 18 and also in Figure 1 show the least-squares means of the values for feed intake (g / piglet / day) by week, obtained from each of the treatment groups.

Table 18: Least-squares means of feed intake (g/ piglet / day) on an as fed basis.

Group	Week 1	Week 4	Mean value week 1 to week 4	Residual Standard Deviation (RSD)
CV 250	206	770^{b}	440^{b}	131.1
CV 500	158	716^{ab}	407^{ab}	131.1
C	187	596^{a}	367^{a}	131.1
C+	324	1318^{c}	832^{c}	131.1
Meat	198	1274^{c}	718^{c}	131.1

a , b, and c means within a column, with a common superscript (or with no superscript) are not significantly different from each other (LSD; p<0.05)

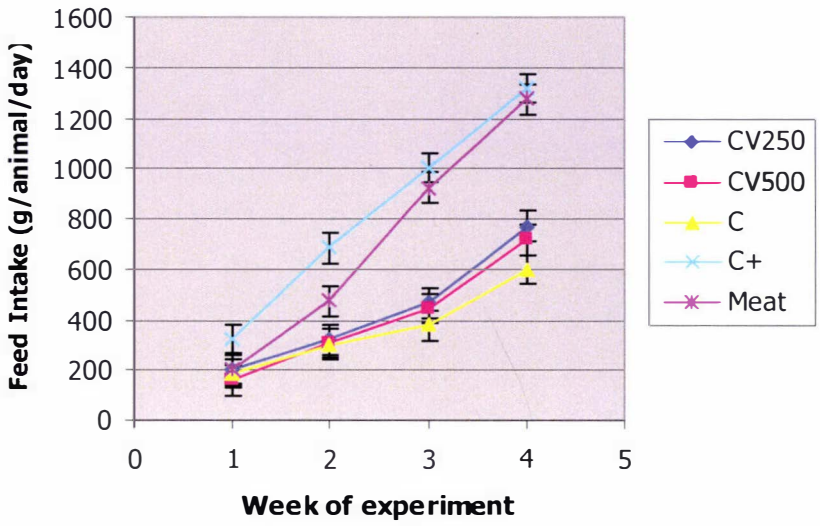


Figure 1: Least-squares means (± S. E) of feed intake (g/ piglet/ day) by week for each of the five treatment groups.

Initially feed intake was not significantly different among the groups of piglets. As the study progressed, changes in intake between the groups became more apparent, with the C+ group consuming significantly more food than the other groups.
Throughout the study the lowest intakes were recorded in groups consuming the C, CV250 and CV500 diets. By week four of the study period feed intakes in the meat and C+ groups were significantly higher than the C, CV250 or CV500. The feed intake of piglets in the C+ group was twice that of the C, CV250 and CV500 groups.

4.2 Growth Rate

Statistically significant differences ($P<0.001$) were observed between the groups and there were also significant differences between weeks, and pigs within groups. There was also a significant interaction between week x group. The results presented in Table 19 and also in Figure 2 show the least- squares means of the values for growth rate (g /piglet/ day) obtained from each of the treatment groups.

Table 19: Least-squares means of growth rate (g/piglet/ day)

Group	Week 1	Week 4	Mean value week 1 to week 4	Residual Standard Deviation (RSD)
CV 250	-40.1 [a b]	172.5 [a]	60.4 [a]	60.4
CV 500	-67.4 [a]	193.5 [a]	52.8 [a]	60.4
C	-35.1 [a b]	174.2 [a]	61.7 [a]	60.4
C+	27.8 [b]	456.2 [b]	218.0 [b]	60.4
Meat	-28.5 [a b]	426.6 [b]	190.2 [b]	60.4

a , b, and c means within a column, with a common superscript (or with no superscript) are not significantly different from each other (LSD; p<0.05)

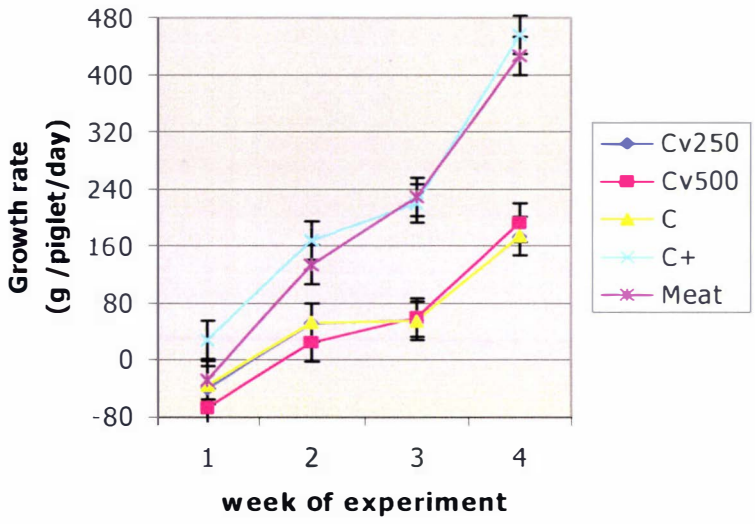


Figure 2: Least-squares means (± S. E) of growth rate (g/day) by week for each of the five treatment groups.

Levels of feed intake determined the rates of growth amongst piglets in all of the groups. The initial poor feed intake resulted in negative rates of growth in four of the five treatment groups. Only piglets in the C+ group reported small positive increases, which were significantly different from other groups. Throughout the study the lowest growth rates were reported in piglets in the C, CV250 and CV500 groups. By week four of the study there were no significant differences between the meat and C+ groups. Both groups had rates of growth greater than the C, CV250 and CV500 groups.

4.3 Haematology

Table 20 the shows the significance of the effects of group, pig within group, week and week x group on a complete red blood cell count (CBC). The r-squared values indicate how much of the variation between the treatments can be explained, by the statistical model shown in section 3.6 (data analysis): Where the CBC characteristics and their abbreviations are listed in Table 14.

Table 20: Significance levels of the effects of group, pigs within group, week and week x group on blood parameters.

	Group	Pig (group)	Week	Week x group	R^2
RBC	***	***	***	***	0.89
HGB	***	***	***	***	0.96
HCT	***	***	***	***	0.95
MCV	***	***	***	***	0.98
MCH	***	***	***	***	0.99
MCHC	***	***	***	*	0.93
CHCM	***	***	***	**	0.93

ns: not significant p>0.05; * p<0.05; ** p< 0.01; *** p<0.001

4.3.1 Red Blood Cells

Statistically significant differences (P< 0.001) were observed between the groups and there were also significant differences between weeks, and pigs within groups. There was also a significant interaction between week x group for red blood cells. The results presented in Table 21 and also in Figure 3 show the least-squares means of the values for red blood cells (x 10^{12} / L) obtained from each of the treatment groups.

Table 21: Least-squares means of red blood cells count (x 10^{12} / L)

Group	Week 0	Week 4	Change from week 0 to week 4	Mean value week 0 to week 4	Residual Standard Deviation (RSD)
Cv250	3.53 [a]	4.74 [a]	+ 1.21 [b]	4.22 [a]	0.47
Cv500	4.01 [a]	5.94 [b]	+ 1.93 [b]	4.92 [b]	0.47
C	3.45 [a]	4.82 [a]	+ 1.37 [b]	4.29 [a]	0.47
C+	5.99 [b]	6.03 [b]	+ 0.04 [a]	6.11 [c]	0.47
Meat	3.91 [a]	5.86 [b]	+ 1.95 [b]	5.10 [b]	0.47

a , b, and c means within a column, with a common superscript (or with no superscript) are not significantly different from each other (LSD; p<0.05)

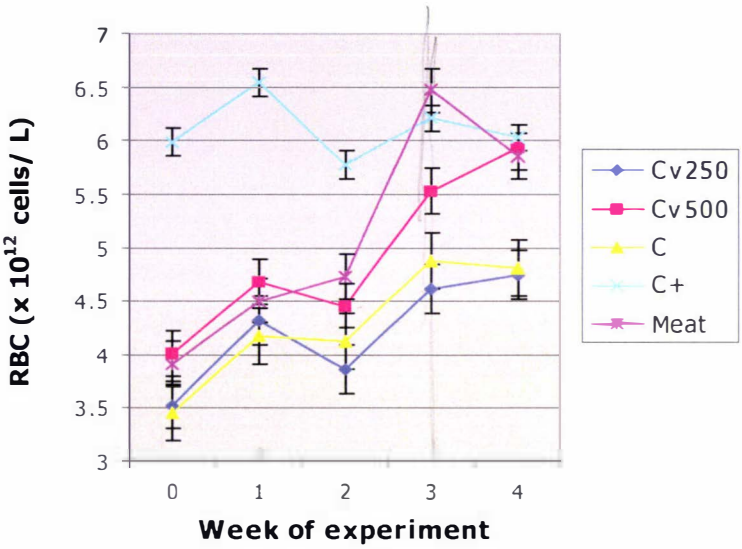


Figure 3: Least-squares means (± S. E) of red blood cells (RBC) x 10^{12} /L by week for each of the five treatment groups.

By design piglets in the C+ group began the study with red blood cell counts that were significantly different from the other groups. This was because the piglets in this group received an injection of supplementary iron shortly after birth, whereas iron was only available to the C, CV250, CV500 and meat groups through their respective diets.

Red blood cell production increased in line with dietary iron intake throughout the duration of the study so that piglets with the highest intakes had the greatest increases. Increases in red blood cell counts were greatest in iron deficient piglets, with piglets in the meat and CV500 groups having red blood cell counts that were significantly different from those piglets consuming either the C or CV250 diets. Little change was observed in the C+ group as these piglets had red blood cell counts that were already within the normal physiological range as shown in Table 5.

4.3.2 Blood Haemoglobin Concentrations (HGB)

Statistically significant differences (P< 0.001) were observed between the groups and there were also significant differences between weeks, and pigs within groups. There was also a significant interaction between week x group. The results presented in Table 22 and also in Figure 4 show the least-squares means of the values for haemoglobin (g / L) obtained from each of the treatment groups.

Table 22: Least square means of blood haemoglobin concentration (g / L).

Group	Week 0	Week 4	Change from week 0 to week 4	Mean value week 0 to week 4	Residual Standard Deviation (RSD)
Cv250	42.0 [a]	46.0 [a]	+ 4 [b]	44.0 [a b]	6.12
Cv500	46.6 [a]	57.0 [b]	+ 10.4 [b]	50.2 [b]	6.12
C	38.2 [a]	42.61 [a]	+ 4.41 [b]	41.2 [a]	6.12
C+	113.4 [b]	101.4 [c]	- 12 [a]	107.7 [d]	6.12
Meat	42.2 [a]	64.4 [b]	+ 22.2 [c]	53.4 [b c]	6.12

a , b, and c means within a column, with a common superscript (or with no superscript) are not significantly different from each other (LSD; p<0.05)

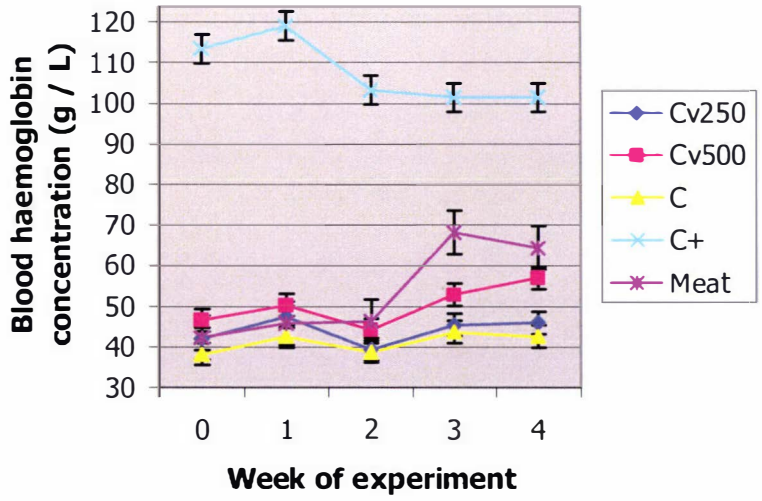


Figure 3: Least-squares means (± S. E) of blood haemoglobin concentration (HGB) g / L by week for each of the five treatment groups.

By design, at the beginning of the study the piglets in the C+ group had blood haemoglobin concentrations that were significantly different from all the other groups. The C+ group reported a small decrease in blood haemoglobin concentrations over the duration of the study, indicative of decreasing iron reserves during a period of increased requirements as the animal grows. Again in the groups containing iron deficient piglets, blood haemoglobin concentrations increased inline with feed intake. The meat group, with the highest feed intake amongst the iron deficient groups had the greatest increases in blood haemoglobin concentration. Overall there were no significant differences between haemoglobin concentrations in the C, CV250 and CV500, despite weekly differences. Even though piglets in the C, Cv250, Cv500 and meat groups saw increases in blood haemoglobin concentrations, at the end of the study haemoglobin concentrations in these animals were still 50% lower than normal physiological values for blood haemoglobin concentration as shown in Table 5.

4.2.3 Hematocrit (HCT)

Statistically significant differences ($P < 0.001$) were observed between the groups and there were also significant differences between weeks, and pigs within groups. There was also a significant interaction between week x group. The results presented in Table 23 and also in Figure 5 show the least-squares means of the values for hematocrit (L / L) obtained from each of the treatment groups.

Table 23: Least-squares means of hematocrit (L / L)

Group	Week 0	Week 4	Change from week 0 to week 4	Mean value week 0 to week 4	Residual Standard Deviation (RSD)
Cv250	0.15 [a]	0.17 [a]	+ 0.02 [b]	0.16 [a b]	0.02
Cv500	0.16 [a]	0.21 [b]	+ 0.05 [c]	0.18 [b]	0.02
C	0.13 [a]	0.15 [a]	+ 0.02 [b]	0.15 [a]	0.02
C+	0.36 [b]	0.34 [c]	- 0.02 [a]	0.35 [b d]	0.02
Meat	0.15 [a]	0.24 [b]	+ 0.09 [d]	0.19 [b c]	0.02

a , b, c and d means within a column, with a common superscript (or with no superscript) are not significantly different from each other (LSD; $p<0.05$)

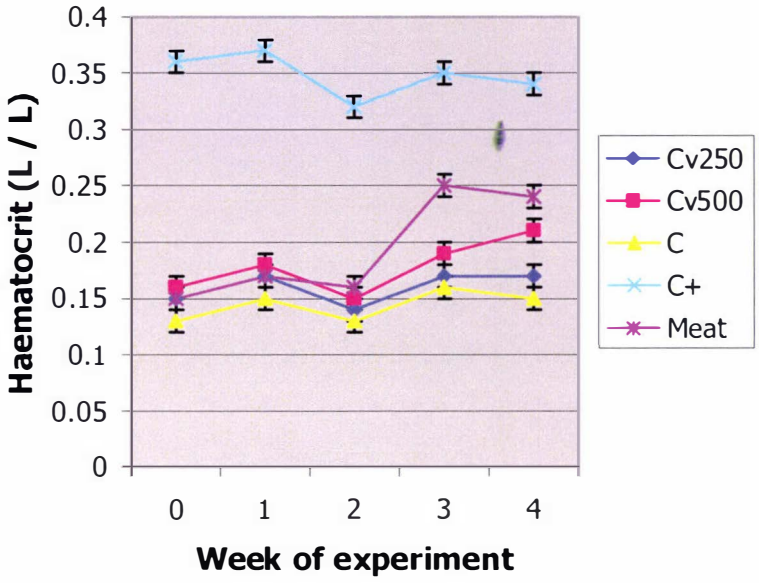


Figure 5: Least-squares means (± S. E) of hematocrit (HCT) L / L by week for each of the five treatment groups.

Haematocrit is an alternative measurement of red blood cell number. It measures the percentage of red cells in whole blood. Therefore reflecting the changes observed in red blood cell counts as well as their size.

4.3.4 Mean Cell Volume (MCV)

Statistically significant effects (P< 0.001) were observed between the groups and also there were significant differences between weeks, and pigs within groups. There was also a significant interaction between week x group. The results presented in Table 24 and also in Figure 6 show the least-squares means of the values for mean cell volume (f L) obtained from each of the treatment groups.

Table 24: Least-squares means of mean cell volume (f L)

Group	Week 0	Week 4	Change from week 0 to week 4	Mean value week 0 to week 4	Residual Standard Deviation (RSD)
Cv250	42.2 [c]	35.2 [b]	- 7.0 [a]	37.5 [b]	1.46
Cv500	39.9 [b]	34.6 [b]	- 5.3 [a]	35.7 [a b]	1.46
C	38.8 [a b]	32.0 [a]	- 6.8 [a]	34.4 [a]	1.46
C+	60.3 [d]	56.2 [d]	- 4.1 [b]	56.8 [c]	1.46
Meat	37.9 [a]	39.9 [c]	+ 2.0 [c]	37.5 [b]	1.46

a , b, c and d means within a column, with a common superscript (or with no superscript) are not significantly different from each other (LSD; $p<0.05$)

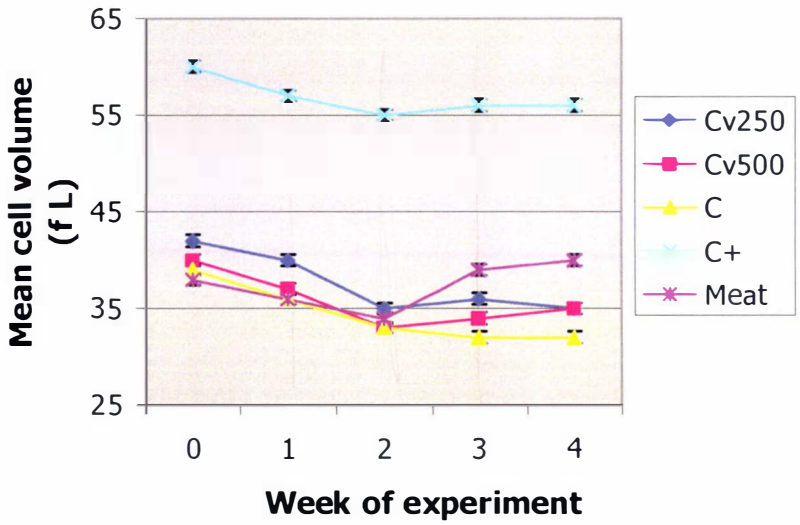


Figure 6: Least-squares means (± S. E) of mean cell volume (MCV) f L by week for each of the five treatment groups.

The mean cell volume of red cells in the C+ group were significantly different from all other groups throughout the study, and also maintained within a narrow range, within normal physiological ranges. Other groups in the study had variable MCV values, which initially declined prior to reaching a plateau. Subsequent increases in the MCV values were then observed in the CV500 and the meat groups. The low MCV values indicate the red blood cells in circulation are small. This is because insufficient iron was available for their manufacture.

4.3.5 Mean Cell Haemoglobin

Statistically significant effects (P< 0.001) were observed between the groups and also there were significant differences between the weeks, and pig within groups. There was also a significant interaction between week x group. The results presented in Table 25 and also in Figure 7 show the least-squares means of the values for mean cell haemoglobin (p g) obtained from each of the treatment groups.

Table 25: Least-squares means of mean cell haemoglobin. (p g)

Group	Week 0	Week 4	Change from week 0 to week 4	Mean value from week 0 to week 4	Residual Standard Deviation (RSD)
Cv250	11.88 [b]	9.60 [b]	- 2.28 [a]	10.45 [b]	0.39
Cv500	11.56 [a b]	9.56 [b]	- 2.0 [a]	10.22 [b]	0.39
C	11.12 [a]	8.87 [a]	- 2.25 [a]	9.47 [a]	0.39
C+	18.98 [c]	16.8 [d]	- 2.18 [a]	17.80 [c]	0.39
Meat	10.8 [a]	10.8 [c]	0 [b]	10.41 [b]	0.39

a , b, and c means within a column, with a common superscript (or with no superscript) are not significantly different from each other (LSD; p<0.05)

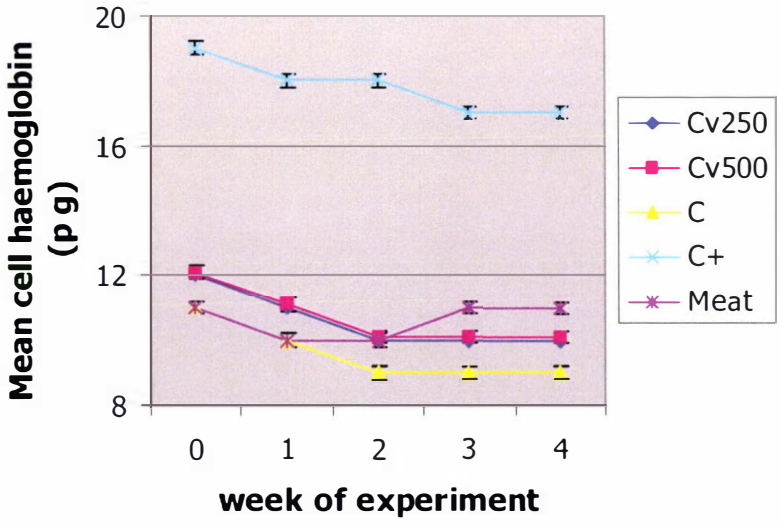


Figure 7: Least-squares means (± S. E) of mean cell haemoglobin (MCH) p g by week for each of the five treatment groups.

As seen in measurements of mean cell volume, mean cell haemoglobin concentrations in the C+ group were significantly different from the others. As the supplementary iron injected shortly after birth would have provided adequate reserves of iron so that each red blood cell manufactured contained an optimal concentration of haemoglobin. Iron deficient piglets had no iron reserves that could be used in the manufacture of red blood cell so that the red cells of these animals were poorly haemoglobinised. As feed intake increased during the last weeks of the study the decline in MCH concentrations ceased. A small increase in the MCH concentration was observed in piglets consuming the meat diet, indicating that meat iron was being more readily assimilated.

4.3.6 Mean corpuscle, haem concentration (MCHC)

Statistically significant differences ($P < 0.001$) were observed between the groups and there were also significant differences between the variables week, and pigs within groups. There was also a significant interaction between week x group for MCHC. The results presented in Table 26 show the least-squares means of the values for MCHC (g / L) obtained from each of the treatment groups.

Table 26: Least-squares means of mean corpuscle, haem concentration (MCHC g / L)

Group	Week 0	Week 4	Change from week 0 to week 4	Mean value from week 0 to week 4	Residual Standard Deviation (RSD)
Cv250	282 [a]	273 [a]	- 9	279 [a]	5.57
Cv500	290 [a]	276 [a]	- 14	286 [b]	5.57
C	286 [a]	277 [a]	- 9	283 [a b]	5.57
C+	314 [b]	299 [b]	-15	313 [c]	5.57
Meat	285 [a]	271 [a]	- 14	278 [a]	5.57

a , b, and c means within a column, with a common superscript (or with no superscript) are not significantly different from each other (LSD; $p<0.05$)

MCHC values in piglets in the C+ group were significantly different from all other groups throughout the study. There were no significant differences between the other groups regardless of diet and iron status.

4.3.7 Corpuscular haemoglobin constant (CHCM)

Statistically significant differences (P< 0.001) were observed between the groups and there were also significant differences between also between weeks, and pigs within groups. There was also a significant interaction between week x group. The results presented in Table 27 show the least square mean of the values for corpuscular haemoglobin constant obtained from each of the treatment groups.

Table 27: Least square means of corpuscular haemoglobin constant (g / L)

Group	Week 0	Week 4	Change from week 0 to week 4	Mean value week 0 to week 4	Residual Standard Deviation (RSD)
Cv250	276.6 [a]	272.6 [a]	- 4.0	275.3 [a]	5.25
Cv500	284.4 [a]	278.4 [a]	- 6.0	282.8 [a]	5.25
C	281.0 [a]	277.5 [a]	- 3.5	279.5 [a]	5.25
C+	313.4 [b]	299.2 [b]	-14.2	312.1 [b]	5.25
Meat	279.4 [a]	273.2 [a]	- 6.2	276.4 [a]	5.25

a , b, and c means within a column, with a common superscript (or with no superscript) are not significantly different from each other (LSD; p<0.05)

CHCM values in piglets in the C+ group were significantly different throughout the study. There was no significant difference between the other groups regardless of diet and iron status.

4.4 Matrix analysis

Table 28 shows the significance of the effects of group, pigs within groups, weeks and week x group on the outcome of red blood cell characteristics, when they were analysed and placed in 3 x 3 matrix (M1 to M9, see Table 15) describing the haemoglobin concentration (HC) and cell volume (V). The r-squared values indicate how much of the variation between the groups can be explained by the statistical model shown in section 3.6 (data analysis):

Table 28: Significance levels of the effects of group, pigs within group, week and week x group on red blood cell characteristics.

	Group	Pig (group)	Week	Week x group	R^2
M1	**	***	***	***	0.73
M2	***	***	***	***	0.88
M3	***	***	***	***	0.92
M4	***	***	***	***	0.93
M5	***	*	**	ns	0.86
M6	***	***	***	***	0.88
M8	*	***	***	ns	0.86
M9	***	***	***	***	0.91

ns: not significant p>0.05; * p<0.05; ** p< 0.01; *** p<0.001

The results presented in Table 29 show the least-squares means of the values for red blood cells characterised as M1-M9 obtained from each of the treatment groups

Table 29: The least-squares means of red blood cell characteristic M1-M9.

		GROUP					
CELL	WEEK	CV250	CV500	C	C+	MEAT	RSD
M1	0	21.4^{ab}	12.0^{a}	30.8^{ab}	64.4^{b}	9.0^{a}	38.9
	4	8.0^{a}	5.6^{a}	6.1^{a}	202.8^{c}	103.6^{b}	38.9
M2	0	13039^{b}	10876^{b}	10231^{b}	4327^{a}	11251^{b}	3370
	4	17474^{b}	20281^{c}	13599^{b}	6929^{a}	27801^{d}	3370
M3	0	4740^{b}	6261^{b}	6871^{b}	9^{a}	7747^{c}	1818
	4	9346^{c}	8890^{c}	11108^{c}	5^{a}	4213^{b}	1818
M4	0	49.6^{b}	29.6^{b}	31.8^{b}	1547.0^{a}	26.4^{b}	147.3
	4	4.6^{b}	2.6^{b}	1.8^{b}	377.0^{a}	10.4^{b}	147.3
M5	0	11786^{a}	14356^{a}	10604^{a}	43736^{b}	11619^{a}	7340
	4	7720^{a}	10199^{a}	6186^{a}	41316^{b}	14704^{a}	7340
M6	0	$1895^{a\,b}$	3415^{b}	3409^{b}	215^{a}	3721^{b}	1468
	4	5436^{b}	9179^{c}	8592^{c}	192^{a}	5076^{b}	1468
M7	0	No cells were identified in this category					
	4						
M8	0	216.0^{b}	314.8^{b}	290.4^{b}	79.0^{a}	281.8^{b}	82.5
	4	271.0^{b}	325.2^{bc}	271.2^{b}	38.6^{a}	204.6^{b}	82.5
M9	0	45.2^{a}	72.2^{b}	87.2^{b}	3.4^{a}	90.8^{b}	36.7
	4	206.4^{c}	219.4^{c}	290.5^{d}	2.4^{a}	92.8^{b}	36.7

a , b, and c means within a column, with a common superscript (or with no superscript) are not significantly different from each other (LSD; p<0.05)

4.4.1 M1.

Statistically significant effects (P< 0.001) were observed between the groups and there were also significant differences between weeks, and pigs within groups. There was also a significant

interaction between week x group. The results presented in Table 29 show the least-squares means of the values for red blood cells characterised as M1 obtained from each of the treatment groups.

4.4.2 M2.

Statistically significant effects (P< 0.001) were observed between the groups and there were also significant differences between weeks, and pigs within groups. There was also a significant interaction between week x group for M2. The results presented in Table 29 show the least-squares means of the values for red blood cells characterised as M2 obtained from each of the treatment groups.

4.4.3 M3.

Statistically significant effects (P< 0.001) were observed between the groups and there were also significant differences between weeks, and pigs within groups. There was also a significant interaction between week x group. The results presented in Table 29 show the least-squares means of the values for red blood cells characterised as M3 obtained from each of the treatment groups.

4.4.4 M4.

Statistically significant effects (P< 0.001) were observed between the groups and there were also significant differences between weeks, and pigs within groups. There was also a significant interaction between week x group. The results presented in Table 29 the least-squares means of the values for red blood cells characterised as M4 obtained from each of the treatment groups.

4.4.5 M5.

Statistically significant effects (P< 0.001) were observed between the groups and there were also significant differences between weeks and pigs within groups. Week x group was not a significant interaction. The results presented in Table 29 show the least-squares means of the values for red blood cells characterised as M5 obtained from each of the treatment groups.

4.4.6 M6.

Statistically significant effects (P< 0.001) were observed between the groups and there were also significant differences between weeks, and pigs within groups. There was also a significant interaction between week x group. The results presented in Table 29 show the least-squares means of the values for red blood cells characterised as M6 obtained from each of the treatment groups.

4.4.7 M7

There were no cells identified in this category

4.4.8 M8.

Statistically significant effects (P< 0.001) were observed between the groups and there were also significant differences between week and pigs within groups. There was not a significant interaction between week x group. The results presented in Table 29 show the least-squares means of the values for red blood cells characterised as M8 obtained from each of the treatment groups.

4.4.9 M9.

Statistically significant effects (P< 0.001) were observed between the groups and also between the variables week, and pigs within groups. There was also a significant interaction between week x group. The results presented in Table 29 show the least-squares means of the values for red blood cells characterised as M9 obtained from each of the treatment groups.

Red cells were classified by volume and haemoglobin concentration using a 3 x 3 matrix. Throughout the study, the classification of red blood cells reflected the iron status of the piglets. Piglets in the C+ group maintained approximately 90% of red blood cells in the optimum m5 position. Whereas the iron deficient piglets had many red cell in the lower matrices categories, indicating that insufficient iron had resulted in the manufacture of red blood cells that were small and had lower concentrations of haemoglobin. The red blood cells have a life span of approximately 120 days therefore during this study, which lasted just 28 days only small changes in red cell classification were evident. By week 4 of the study piglets consuming the meat diet

increased the number of red blood cells classified as m5. Where as the number of red cells in this category decreased for C, CV250, and CV500 groups.

Figures 8 and 9 show the red blood cell characteristics at the beginning and again at the completion of the experiment.

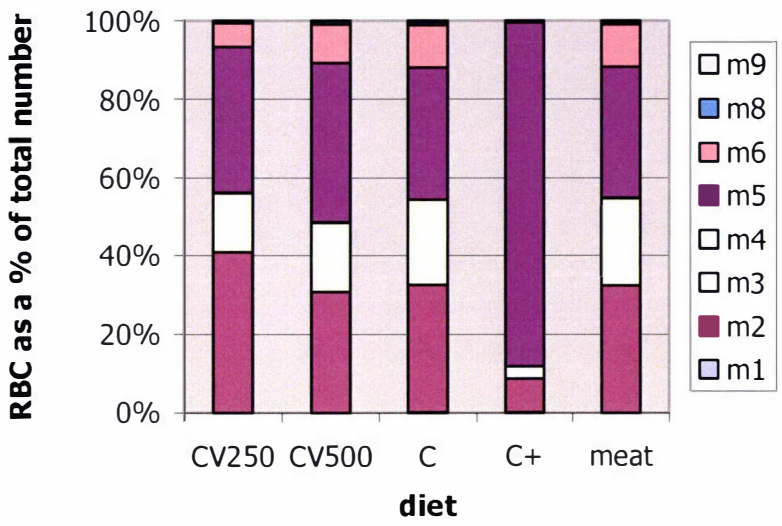


Figure 8: Least-squares means (± S. E) of red blood cell characteristic (volume and haemoglobin concentration) as a percentage of the total for each of the five treatment groups at the beginning of the trial.

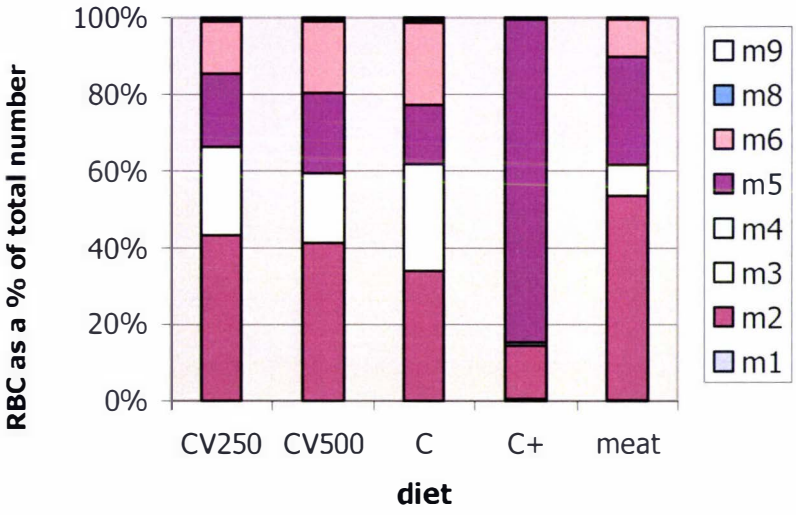


Figure 9: Least-squares means (± S. E) of red blood cell characteristic (volume and haemoglobin concentration) as a percentage of the total for each of the five treatment groups at the end of the trial.

4.5 Serum Iron Concentration.

Table 30 shows the significance of the effects of group, pigs within groups, week and week x group on serum iron and iron binding proteins. The r-squared values indicate how much of the variation between the treatments can be explained by the statistical model shown in section 3.6 (data analysis):

Table 30: Significance level of the effects of group, pigs within group, week and week x group on serum iron and iron binding proteins

	Group	Pig (group)	Week	Week x group	R^2
Iron	***	Ns	ns	ns	0.70
UIBC	***	Ns	***	ns	0.77
HGB Fe	***	***	***	***	0.91

ns: not significant p>0.05; * p<0.05; ** p< 0.01; *** p<0.001

Statistically significant differences (P< 0.001) were observed between the groups. There were no significant differences between weeks, pig within groups and week x group. The results presented in Table 31 and also in Figure 10 show the least-squares means of the values for total serum iron obtained from each of the treatment groups.

Table 31: The least-squares means of serum iron concentration (μmol / L)

Group	Week 0	Week 4	Change from week 0 to week 4	Mean value week 0 to week 4	Residual Standard Deviation (RSD)
Cv250	1.2[a]	3.0[a]	+ 1.8[a]	1.9[a]	6.63
Cv500	1.1[a]	2.3[a]	+ 1.2[a]	1.3[a]	6.63
C	1.1[a]	1.5[a]	+ 0.4[a]	1.3[a]	6.63
C+	10.2[b]	22.2[b]	+ 12[b]	19.0[b]	6.63
Meat	0.8[a]	6.8[a]	+ 6[a]	2.7[a]	6.63

a , b, and c means within a column, with a common superscript (or with no superscript) are not significantly different from each other (LSD; p<0.05)

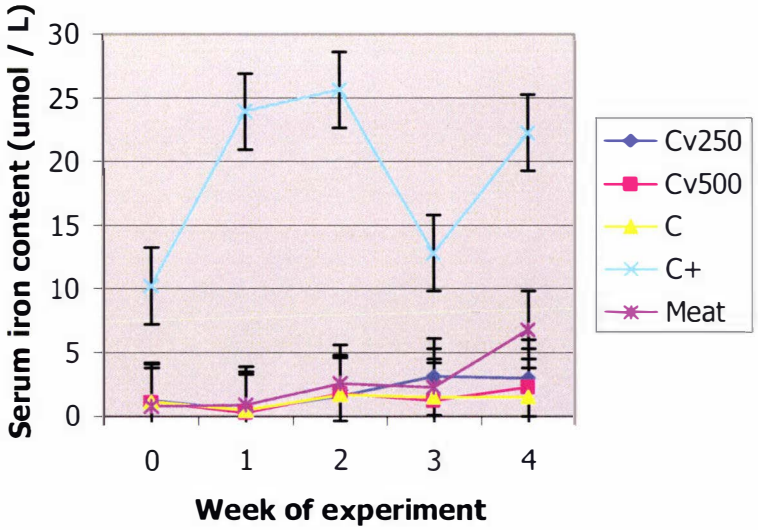


Figure 10: Least-squares means (± S. E) of serum iron values (μmol / L) for each of the treatment groups

.

By design, Serum iron concentrations were highest in the C+ group. This reflected the difference in the administration of supplementary iron (as shown in Table 8). Initially there were no significant differences between the serum iron concentrations of the other four groups. The serum iron concentration increased across all groups throughout the duration of the study. Of the iron deficient piglets, the meat group showed the largest increases, which may be a result of differences in the bioavailability of the different forms of iron found between the diets.

4.5.1 Unsaturated iron binding capacity (UIBC)

Statistically significant effects ($P < 0.001$) were observed between the groups and there were also significant differences between weeks. The variables pigs within groups and the interaction between week x group were not significant. The results presented in Table 32 and also in Figure 10 show the least-squares means of the values for unsaturated iron binding capacity obtained from each of the treatment groups.

Table 32: The least-squares means of unsaturated iron binding capacity (μmol / L).

Group	Week 0	Week 4	Change from week 0 to week 4	Mean value week 0 to week 4	Residual Standard Deviation (RSD)
Cv250	159.4 [b]	130.6 [b]	- 28.8	128 [b]	23.4
Cv500	163.8 [b]	132.8 [b]	- 31.0	125 [b]	23.4
C	158.8 [b]	137.8 [b]	- 21.0	129 [b]	23.4
C+	79.0 [a]	69.6 [a]	- 9.4	61 [a]	23.4
Meat	168.0 [b]	142.8 [b]	- 25.2	139 [b]	23.4

a , b, and c means within a column, with a common superscript (or with no superscript) are not significantly different from each other (LSD; $p<0.05$)

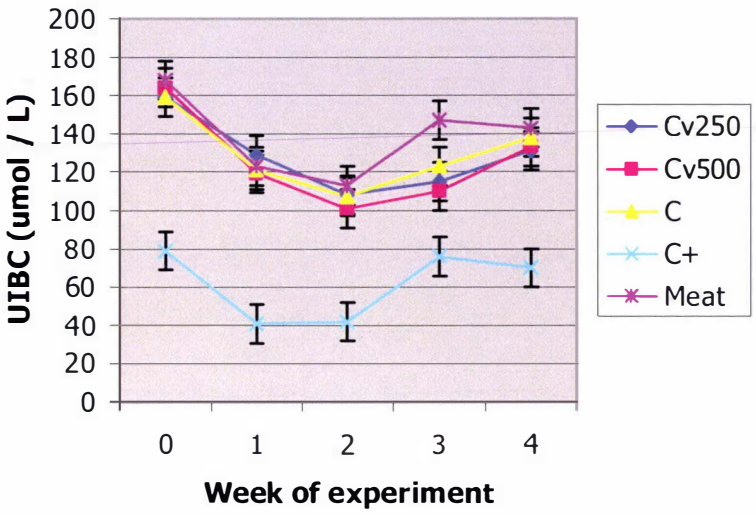


Figure 11: Least- squares means (± S. E) of unsaturated iron binding capacity (μmol / L) for each of the treatment groups.

Unsaturated iron binding capacities reflected the difference in iron status between the groups. The iron deficient piglets had higher UIBC levels than the non-iron deficient piglets indicating that there were many unfilled binding sites on the iron binding proteins (mainly transferrin) in the blood of these animals.

4.6 Iron Retention

The iron retention efficiency was calculated as the slope of the relationship between iron intake and body HBG Fe (see page 44). The results presented in Table 33 and also in Figure 12 show the least-squares means of the values for body HGB Fe obtained from each of the treatment groups. Statistically significant effects ($P < 0.001$) were observed between the groups and there were also significant differences between weeks and between pigs within groups. There was also a significant interaction between week x group.

Table 33: The least-squares means of body haemoglobin iron content (mg) by week for each of the treatment groups.

Group	Week 0	Week 4	Change from week 0 to week 4	Mean value week 0 to week 4	Residual Standard Deviation (RSD)
Cv250	52.8 [a]	78.4 [a]	+25.6 [a]	60.33 [a b]	23.7
Cv500	57.8 [a]	91.1 [a]	+34.0 [a]	64.64 [a b]	23.7
C	49.3 [a]	76.3 [a]	+26.9 [a]	56.81 [a]	23.7
C+	146.3 [b]	278.0 [c]	+131.7 [b]	188.23 [c]	23.7
Meat	53.4 [a]	191.9 [b]	+138.5 [b]	87.35 [b]	23.7

a , b, and c means within a column, with a common superscript (or with no superscript) are not significantly different from each other (LSD; p<0.05)

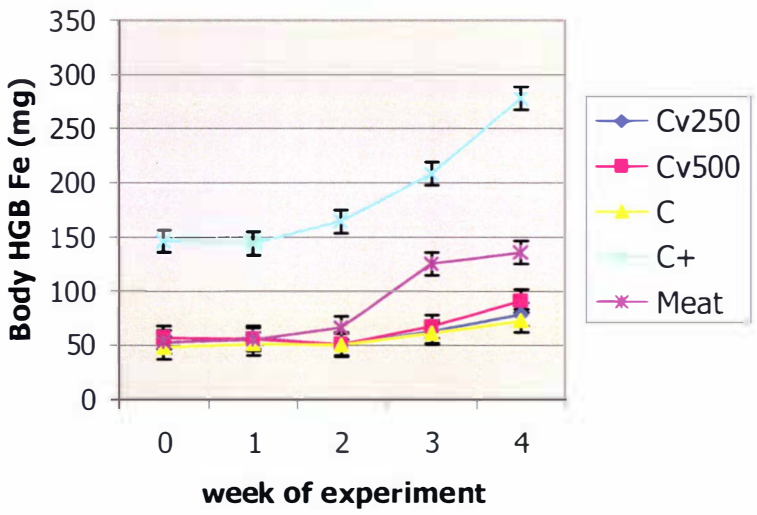


Figure 12: Least-squares means (± S. E) of body Haemoglobin iron (mg) for each of the treatment groups.

The regression analysis statistics of body HGB Fe x FEI are shown below.

	C	CV250	CV500	Meat	C+	SE
Intercept (a)	48.7[a]	50.6a	51.3[a]	46.9[a]	141.3[b]	4.90
Slope (b)	0.032[a]	0.039[a]	0.067[a]	0.169[b]	0.126[b]	0.019

a , b, and c means within a column, with a common superscript (or with no superscript) are not significantly different from each other (LSD; p<0.05)

Body haemoglobin iron accumulated more rapidly in iron deficient piglets consuming the meat diet than iron deficient piglets consuming diets containing non-meat iron. This indicates the differences in bioavailability between meat and non-meat sources of iron and also their ability to return iron deficient piglets back to haematologic normality more rapidly than non-meat sources of iron. Vitamin C was not significant in returning iron deficient subject back to haematologic normality.

4.7 White blood cells (WBC)

White blood cells combat pathogens and other foreign substances that enter the body.
Table 34 shows the significance of group, pigs within groups, weeks and week x group on differential white blood cell evaluations. The r-squared values indicate how much of the variation between the treatments can be explained by the statistical model shown in section 3.6 (data analysis).

Table 34: The significance of dietary treatment on a differential white blood cell count.

	Group	Pig (group)	Week	Week x group	R^2
White blood cells	ns	***	***	ns	0.68
Neutrophil cells	ns	***	***	ns	0.64
Lymphocyte cells	ns	***	***	ns	0.73
Monocyte cells	ns	***	**	ns	0.57
Eosinophil cells	**	**	**	ns	0.61
Basophil cells	ns	***	***	ns	0.64

ns: not significant p>0.05; * p<0.05; ** p< 0.01; *** p<0.001

Statistically significant differences (P< 0.001) were observed between pigs within groups and weeks. There were no significant differences between the groups or no significant interactions between week x group. The results presented in Table 35 and also in Figure 13 show the least-squares means of the values for white blood cells obtained from each of the treatment groups.

Table 35: The least-squares means values for white blood cells (10^9 cells / L).

Group	Week 0	Week 4	Change from week 0 to week 4	Mean value week 0 to week 4	Residual Standard Deviation (RSD)
Cv250	8.4	17.8 [a]	+ 9.4 [a]	13.3	4.98
Cv500	12.4	17.7 [a]	+ 5.3 [a]	12.5	4.98
C	11.7	15.1 [a]	+ 3.4 [a]	12.6	4.98
C+	12.0	17.7 [a]	+ 5.7 [a]	16.8	4.98
Meat	10.7	22.7 [b]	+12.0 [b]	17.6	4.98

a , b, and c means within a column, with a common superscript (or with no superscript) are not significantly different from each other (LSD; $p<0.05$)

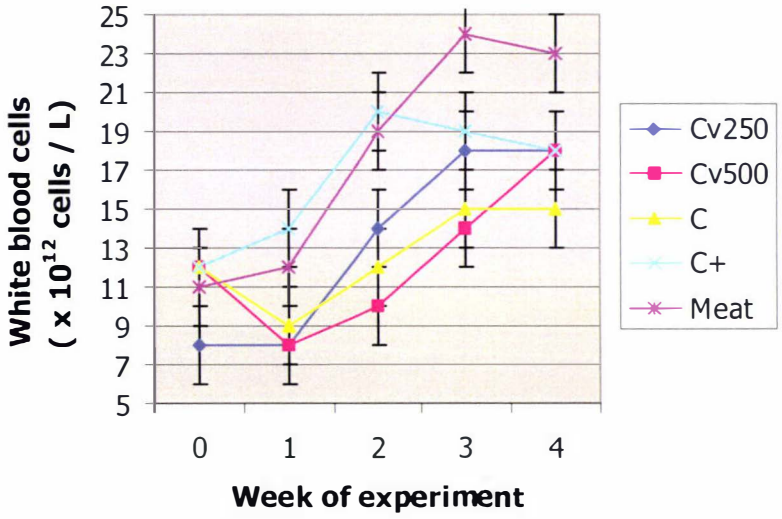


Figure 13: Least-squares means (± S. E) of white blood cell volume x 10^9 / L by week for each of the five treatment groups

A piglet weaned at 21 days is particularly vulnerable to infection, as the levels of passive immunity are declining and the animal's own immune system is not yet mature. (English et al 1984). White blood cells combat pathogens and other foreign substances that enter the body. White blood cell values for weaner piglets are normally between 10-23 x 10^9 cells / L. Initially all animals had white blood cell counts that were within a narrow range of each other, regardless of treatment group. The stress associated with iron deficiency, weaning, and transportation, individual housing and dietary changes predisposed the animals to infection. Day 6+ saw an increase in observed instance of scouring, vomiting and sneezing in all groups. This

corresponded to an elevation in white blood cell counts, neutrophil, lymphocyte, monocyte and basophil cells in individual pigs and at specific periods during the study.
Group was significant in the increasing number of eosinophil cells. These types of cells are produced and increased in number by the body to combat allergens (Totara 1996). This suggests that the allergen may have been a basal feed ingredient of the meat diet.

4.7.1 Neutrophil cells

Statistically significant differences (P< 0.001) were observed between individual pigs within diets and weeks. Dietary treatments and week x diet were not significant. The results presented in Table 36 show the least-squares means of the values for neutrophil cells obtained from each of the treatment groups.

Table 36: The least-squares means values for neutrophil cells 10^9 cells / L.

Group	Week 0	Week 4	Change from week 0 to week 4	Mean value week 0 to week 4	Residual Standard Deviation (RSD)
Cv250	2.06	5.56 [a]	+ 3.5 [a]	3.66	3.24
Cv500	2.18	4.88 [a]	+ 2.7 [a]	2.55	3.24
C	2.14	4.30 [a]	+ 2.16 [a]	2.80	3.24
C+	4.18	4.22 [a]	+ 0.04 [a]	4.66	3.24
Meat	2.78	8.66 [b]	+ 5.88 [b]	6.40	3.24

a , b, and c means within a column, with a common superscript (or with no superscript) are not significantly different from each other (LSD; p<0.05)

4.7.2 Lymphocytes.

Statistically significant differences (P< 0.001) were observed between pigs within diets and weeks. There were no significant differences in dietary treatment or significant interaction between week x diet. The results presented in Table 37 show the least-squares means of the values for lymphocyte cells obtained from each of the treatment groups.

Table 37: The least-squares means values for lymphocyte cells (10^9 cells / L).

Group	Week 0	Week 4	Change from week 0 to week 4	Mean value week 0 to week 4	Residual Standard Deviation (RSD)
Cv250	5.64 [a]	10.42	+ 4.78 [b]	8.45	2.0
Cv500	8.88 [b]	10.98	+ 2.1 [a]	8.78	2.0
C	8.46 [b]	9.49	+ 1.03 [a]	8.73	2.0
C+	6.48 [a]	11.54	+ 5.06 [b]	10.35	2.0
Meat	6.64 [a]	11.52	+ 4.88 [b]	9.48	2.0

a , b, and c means within a column, with a common superscript (or with no superscript) are not significantly different from each other (LSD; p<0.05)

4.7.3 Monocytes.

Statistically significant differences (P< 0.001) were observed between pigs within diets and weeks. There were no statistically significant differences between dietary treatments and the week x diet interactions were not significant. The results presented in Table 38 show the least-squares means of the values for monocyte cells obtained from each of the treatment groups.

Table 38: The least-squares means values for monocyte cells (10^9 cells / L).

Group	Week 0	Week 4	Change from week 0 to week 4	Mean value week 0 to week 4	Residual Standard Deviation (RSD)
Cv250	0.28 [a]	0.74 [a]	+ 0.46	0.5	0.43
Cv500	0.82 [a]	1.10 [a]	+ 0.28	0.7	0.43
C	0.64 [a]	0.60 [a]	-0.04	0.5	0.43
C+	0.60 [a]	1.0 [a]	+ 0.40	0.9	0.43
Meat	0.90 [b]	1.34 [b]	+ 0.44	0.9	0.43

a , b, and c means within a column, with a common superscript (or with no superscript) are not significantly different from each other (LSD; p<0.05)

4.7.4 Eosinophils

Statistically significant differences (P< 0.001) were observed between the dietary treatments, and there were also statistically significant differences between pigs within diets and weeks. There was no significant interaction between week x diet. The results presented in Table 39 show the least-squares means of the values for eosinophil cells obtained from each of the treatment groups.

Table 39: The least-squares means values for Eosinophils cells (10^9 cells / L).

Group	Week 0	Week 4	Change from week 0 to week 4	Mean value week 0 to week 4	Residual Standard Deviation (RSD)
Cv250	0.00 [a]	0.12 [a]	+ 0.12 [b]	0.05 [a]	0.13
Cv500	0.04 [a]	0.16 [a]	+ 0.12 [b]	0.06 [a]	0.13
C	0.02 [a]	0.04 [a]	+ 0.02 [a]	0.02 [a]	0.13
C+	0.34 [b]	0.22 [b]	- 0.12 [a]	0.26 [b]	0.13
Meat	0.00 [a]	0.30 [b]	+ 0.30 [c]	0.12 [a b]	0.13

a , b, and c means within a column, with a common superscript (or with no superscript) are not significantly different from each other (LSD; $p<0.05$)

4.7.5 Basophil cells.

Statistically significant differences ($P< 0.001$) were observed between pigs within diets and week. There were no significant differences between diet, and the week x diet interaction was not significant. The results presented in Table 40 show the least-squares means of the values for basophil cells obtained from each of the treatment groups.

Table 40: The least-squares means values for basophil cells (10^9 cells / L).

Group	Week 0	Week 4	Change from week 0 to week 4	Mean value week 0 to week 4	Residual Standard Deviation (RSD)
Cv250	0.04	0.16 [a]	+ 0.12 [a]	0.09	0.09
Cv500	0.12	0.24 [a]	+ 0.12 [a]	0.11	0.09
C	0.06	0.23 [a]	+ 0.17 [a]	0.12	0.09
C+	0.08	0.24 [a]	+ 0.16 [a]	0.18	0.09
Meat	0.12	0.36 [b]	+ 0.24 [b]	0.19	0.09

a , b, and c means within a column, with a common superscript (or with no superscript) are not significantly different from each other (LSD; $p<0.05$)

Chapter 5

5. Results (experiment 2).

5.1 Intake

Table 41 shows the significance of the effects of group, pigs within groups, weeks and week x group on feed intake and growth rate. The effects of sex, sex x group, and pig (sex x group) were also tested in experiment 2.

Sex, as the groups contained both male and female piglets, sex x group; to test for the existence of an interaction as a result of either sex and grouping and pig (sex x group); where pigs within groups were tested for any interaction which may exist as a result of sex differences.

The r-squared values indicate how much of the variation between the groups can be explained, by the statistical model.

Table 41: Significance leves of dietary treatment on feed intake and growth rate

	Group	Sex	Sex x group	Pig (sex x group)	Week	Week x group	R^2
Feed intake	ns	Ns	ns	***	***	ns	0.97
Growth rate	ns	Ns	ns	**	***	ns	0.90

ns: not significant p>0.05; * p<0.05; ** p< 0.01; *** p<0.001

Statistically significant effects (P< 0.001) were observed between pigs within groups and also weeks. There were no significant differences between groups and between sexes. There were also no interactions between week x group and between sex x group. The results presented in Table 42 and also in Figure 14 show the least-squares means of the values for feed intake (g/day) obtained from each of the treatment groups.

Table 42: Least-squares means of feed intake (g/day)

Group	Week 1	Week 4	Change from week 1 to week 4	Mean value week 1 to week 4	Residual Standard Deviation (RSD)
C	466 [a]	1067	+ 601 [b]	823.7 [a]	90.1
C+	655 [b]	1143	+ 488 [a]	950.2 [b]	90.1
M60	579 [b]	1170	+ 591 [a]	939.3 [b]	90.1
M90	455 [a]	1171	+ 716 [c]	911.4 [a b]	90.1
Mr	593 [b]	1167	+ 574 [a]	945.1 [b]	90.1

a , b, and c means within a column, with a common superscript (or with no superscript) are not significantly different from each other (LSD; $p<0.05$)

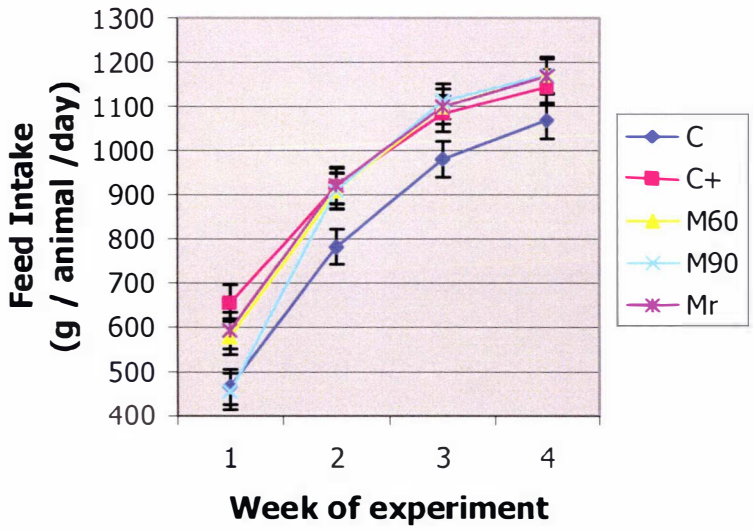


Figure 14: The least-squares means of feed intake (g/day) by week for each of the five treatment groups.

The initial differences between feed intakes in the piglets occurred as diet was changed over from the starter diet, fed during the acclimatisation period, to the experimental diets containing raw or cooked meat or the inorganic source of non-haem iron. Piglets in the C+, M60 and Mr groups had significantly better intakes during the transition. By week four of the study there were no significant differences between the groups. The significance of the changes in feed consumption from week 1 to week 4 was due partly to the initial differences.

5.2 Growth rate

Statistically significant effects (P< 0.001) were observed between pigs within groups and also between weeks. There were no significant differences between groups and between sexes. There were also no significant interactions between week x group and between sex x group. The results presented in Table 43 and also in Figure 15 show the least-squares means of the values for growth rate (g/piglet/day) obtained from each of the treatment groups.

Table 43: Least-squares means of growth rate (g/piglet/day)

Group	Week 1	Week 4	Change from week 1 to week 4	Mean value week 1 to week 4	Residual Standard Deviation (RSD)
C	57 [a]	356 [a b]	+ 299 [b c]	250.4	58.6
C+	163 [b c]	407 [b c]	+ 244 [a b]	311.8	58.6
M60	134 [b]	332 [a]	+ 198 [a]	255.9	58.6
M90	34 [a]	367 [a c]	+ 334 [c]	241.4	58.6
Mr	80 [a b]	332 [a]	+ 253 [a b]	248.7	58.6

a , b, and c means within a column, with a common superscript (or with no superscript) are not significantly different from each other (LSD; p<0.05)

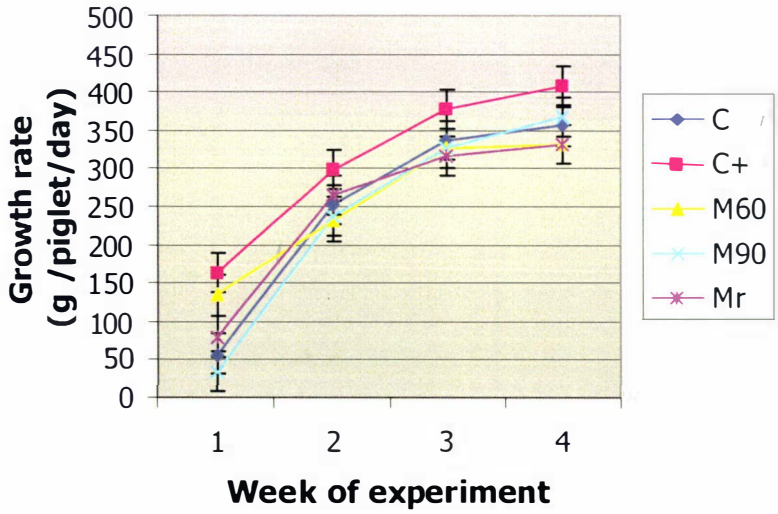


Figure 15: The least-squares means of growth rate (g/piglet/day) by week for each of the five treatment groups.

Feed intakes shaped growth rates throughout the study. Small weekly differences occurred between the groups, but the mean rates of growth were not significantly different between groups.

5.3 Haematology

Table 44 shows the significance of the effects of group, sex, sex x group, pigs within groups, weeks, and week x group on a complete red blood cell count (CBC).
The r-squared values indicate how much of the variation between the groups can be explained by the statistical model, shown in section 3.6 (data analysis): Where the CBC characteristics and their abbreviations listed in are those listed in Table 14.

Table 44: Significance levels of dietary treatment on complete blood count.

	Group	Sex	Sex x group	Pig (sex x group)	Week	Week x group	R^2
RBC	***	ns	ns	*	***	***	0.86
HGB	**	ns	ns	***	***	***	0.90
HCT	*	ns	ns	***	***	***	0.87
MCV	***	ns	ns	***	***	***	0.97
MCH	***	ns	ns	***	***	***	0.97
MCHC	*	ns	ns	***	***	ns	0.85
CHCM	*	ns	ns	***	***	***	0.86

ns: not significant p>0.05; * p<0.05; ** p< 0.01; *** p<0.001

5.3.1 Red blood cells

Statistically significant effects (P< 0.001) were observed between the groups and there were also significant differences between pigs within groups, and between weeks. There was also a significant interaction between week x group. Sex, and the interaction between Sex x group was not significant in the number of red blood cells. The results presented in Table 45 and also in Figure 16 show the least-squares means of the values for red blood cells (x 10.e9/ L) obtained from each of the treatment groups.

Table 45: Least-squares means of red blood cells (x 10.e9/L)

Group	Week 0	Week 4	Change from week 0 to week 4	Mean value week 0 to week 4	Residual Standard Deviation (RSD)
C	5.0	6.4 [a]	+1.4 [a]	5.5 [a]	0.56
C+	5.4	6.7 [a]	+1.3 [a]	6.3 [b]	0.56
M60	5.2	8.7 [c]	+3.5 [c]	7.1 [c d]	0.56
M90	4.81	8.2 [c]	+3.4 [c]	6.7 [b c]	0.56
Mr	4.90	7.4 [b]	+2.5 [b]	6.4 [b c]	0.56

a , b, and c means within a column, with a common superscript (or with no superscript) are not significantly different from each other (LSD; $p<0.05$)

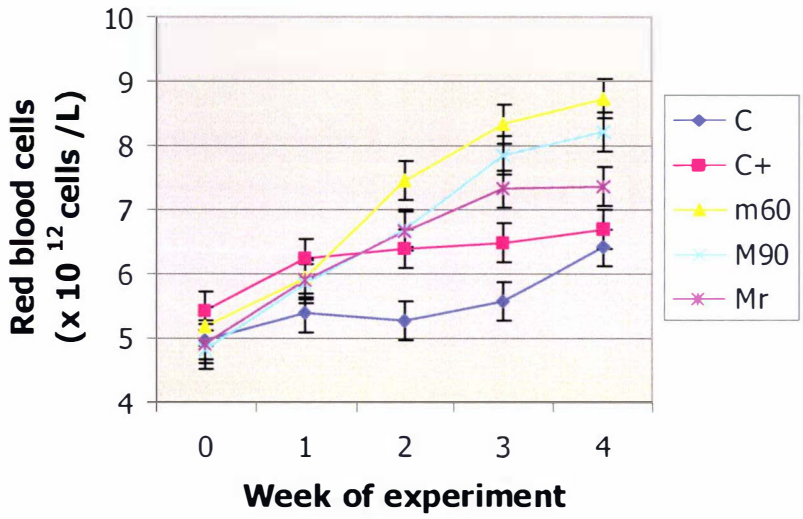


Figure 16: The least-squares means of red blood cells ($x10^{12}$/L) by week for each of the five treatment groups

Blood sampling revealed the initial number of red blood cells was highest in the C+ group. This reflected the differences in level of iron administered shortly after birth (200 mg versus 60 mg) but they were not significantly different from the other groups.

As the study progressed, the number of red blood cells increased across all groups indicating active erythropoiesis was taking place, as dietary iron was assimilated. The variation in the rates of erythropoiesis can in essence be attributed to differences in iron status and iron bioavailability. Not only between haem and non-haem iron as shown in the initial study, but also the effect of processing on haem iron. So that at the end of the study the m60 and m90 groups had significantly higher red blood cell counts than the mr group. Which in tum was significant from

either the C or C+ group, but all groups had red blood cell counts that were within normal physiologically accepted levels (as shown in Table 5).

5.3.2 Blood haemoglobin concentrations (HGB)

Statistically significant effects (P< 0.001) were observed between the dietary groups and there were also significant differences between pigs within diets, and between weeks There was also a significant interaction between week x group. Sex and the interaction between sex x group was not significant in blood haemoglobin concentration. The results presented in Table 46 and also in Figure 17 show the least square mean of the values for blood haemoglobin concentration (g /L) obtained from each of the treatment groups.

Table 46: Least- squares means of haemoglobin (g / L)

Group	Week 0	Week 4	Change from week 0 to week 4	Mean value week 0 to week 4	Residual Standard Deviation (RSD)
C	60.5 $^{a\,b}$	59.1 ^{a}	-1.4 ^{b}	56.4 ^{a}	6.81
C+	97.1 ^{c}	79.3 ^{b}	-17.8 ^{a}	90.6 ^{c}	6.81
M60	59.7 $^{a\,b}$	89.3 ^{c}	+29.6 ^{d}	73.0 ^{b}	6.81
M90	57.9 ^{a}	87.5 $^{b\,c}$	+29.6 ^{d}	71.9 ^{b}	6.81
Mr	66.5 ^{b}	85.1 $^{b\,c}$	+18.6 ^{c}	76.9 ^{b}	6.81

a , b, c and d means within a column, with a common superscript (or with no superscript) are not significantly different from each other (LSD; p<0.05)

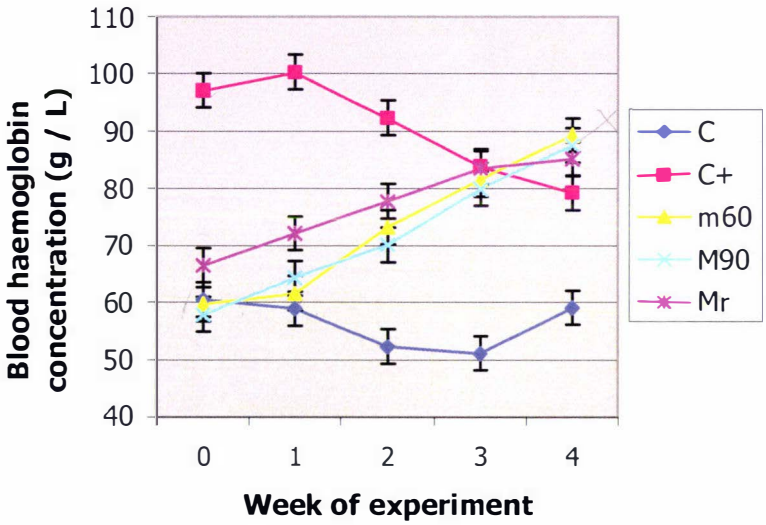


Figure 17: The least-squares means of blood haemoglobin concentration (g /L) by week for each of the five treatment groups.

Having receiving 200 mg iron shortly after birth, piglets in the C+ group began the study with blood haemoglobin concentrations that were significant from the other groups. In spite of that, piglets in this group saw an overall decrease in blood haemoglobin concentration. This was in contrast to piglets in the other groups who increased blood haemoglobin concentration. With the exception was the C group whose haemoglobin concentration overall, remained unchanged. Despite these increases blood haemoglobin concentrations remained below optimal levels.

5.3.3 Hematocrit

Statistically significant effects (P< 0.001) were observed between the groups and there were also significant differences between pigs within groups, and between weeks. There was also a significant interaction between week x group. Sex, and the interaction between sex x group were not significant in the level of haematocrit. The results presented in Table 47 and also in Figure 18 show the least-squares means of the values for hematocrit (L / L) obtained from each of the treatment groups.

Table 47: Least-squares means of hematocrit (L / L)

Group	Week 0	Week 4	Change from week 0 to week 4	Mean value week 0 to week 4	Residual Standard Deviation (RSD)
C	0.21 [a b]	0.20 [a]	-0.01 [b]	0.19 [a]	0.02
C+	0.31 [c]	0.26 [b]	-0.05 [a]	0.29 [c]	0.02
M60	0.21 [a b]	0.30 [c]	+0.09 [d]	0.25 [b]	0.02
M90	0.20 [a]	0.29 [c]	+0.09 [d]	0.25 [b]	0.02
Mr	0.23 [b]	0.29 [c]	+0.06 [c]	0.26 [b]	0.02

a , b, and c means within a column, with a common superscript (or with no superscript) are not significantly different from each other (LSD; $p<0.05$)

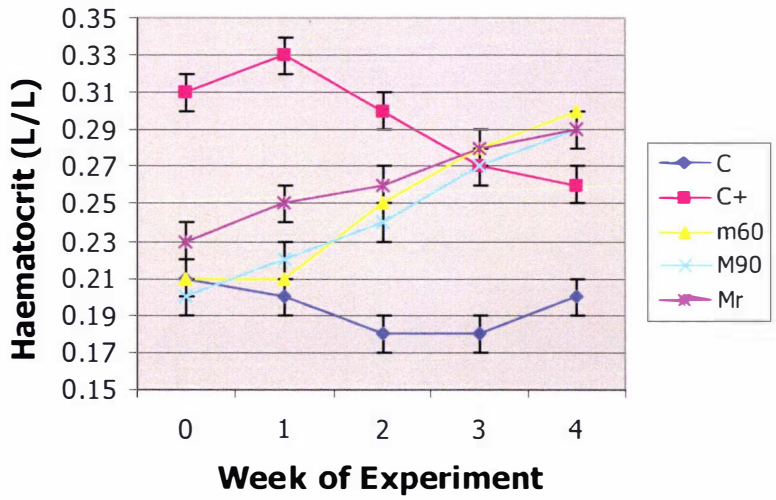


Figure 18: The least-squares means of hematocrit (L /L) by week for each of the five treatment groups.

As haematocrit is an alternative measurement to the number of red blood cells it is not surprising that similar patterns in the number of red blood cell per volume of blood were evident. At the beginning of the study, haematocrit levels in the C+ group were significantly different from the C, m60, m90, and mr groups, but the haematocrit level decreased as the study progressed. In contrast haematocrit levels in the m60, m90 and mr groups increased indicating active erythropoiesis was taking place and as a result the proportion of red blood cell in the blood was also increasing, as dietary iron was assimilated. The variation in the proportion of rates of erythropoiesis was in essence being attributed to differences in iron status and iron bioavailability. So again this is also the reason for differences in the level of haematocrit.

5.3.4 Mean cell volume

Statistically significant effects (P< 0.001) were observed between the groups and there were also significant differences between pigs within groups, and between weeks. There was also a significant interaction between week x group. Sex and the interaction between sex and group were not significant in the mean cell volume. The results presented in Table 48 and also in Figure 19 show the least squares means of the values for mean cell volume (f L) obtained from each of the treatment groups.

Table 48: Least-s uares means of mean cell volume (f L)

Group	Week 0	Week 4	Change from week 0 to week 4	Mean value week 0 to week 4	Residual Standard Deviation (RSD)
C	43.1 [b]	31.8 [a]	-11.3 [b]	35.5 [a]	1.36
C+	58.0 [d]	39.6 [d]	-18.4 [a]	47.7 [d]	1.36
M60	40.7 [a]	34.4 [b]	-6.3 [c]	35.6 [a]	1.36
M90	42.6 [a]	36.3 [c]	-6.3 [c]	37.5 [b]	1.36
Mr	46.0 [c]	39.1 [d]	-6.9 [c]	41.1 [c]	1.36

a , b, and c means within a column, with a common superscript (or with no superscript) are not significantly different from each other (LSD; p<0.05)

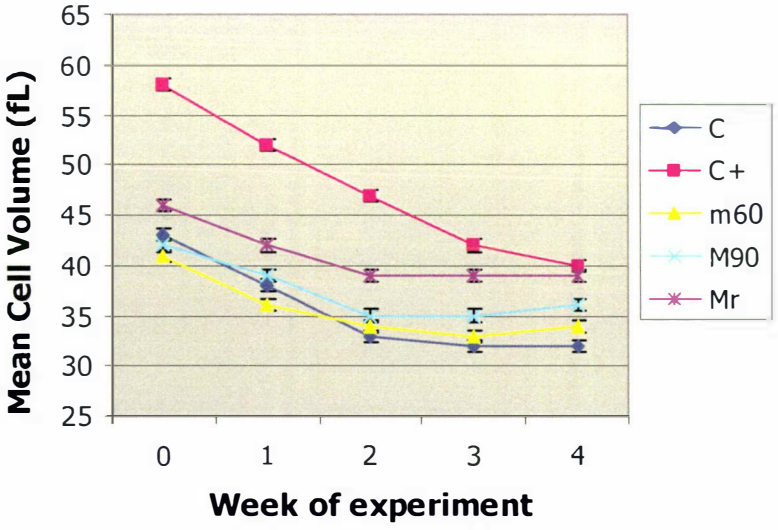


Figure 19: The least square means of mean cell volume (f L) by week for each of the five treatment groups

The data shows that C+ group had mean cell volume that was significantly different from the other treatment groups throughout the study. This despite a reduction in the volume of the red blood cells of 18.4 fl. The other treatment groups also had decreasing mean cell volume values for the first two-weeks of the study until cell volumes reached a plateau. Despite improvements in iron status the piglets in the m60, m90, mr and c groups are still iron deficient. The mean cell volume values obtained suggests that the red cell mass contains large numbers of immature reticulytes that are smaller than mature erythrocytes.

5.3.5 Mean cell haemoglobin

Statistically significant effects (P< 0.001) were observed between the groups and there were also significant differences between pigs within groups, and between weeks. There was also a significant interaction between week x group. Sex and the interaction between sex and group were not significant in the mean cell haemoglobin concentration. The results presented in Table 49 and also in Figure 20 show the least-squares means of the values for mean cell haemoglobin (p g) obtained from each of the treatment groups.

Table 49: Least-squares means of mean cell haemoglobin (p g)

Group	Week 0	Week 4	Change from week 0 to week 4	Mean value week 0 to week 4	Residual Standard Deviation (RSD)
C	12 ^{a}	9 ^{a}	-3 ^{b}	10 ^{a}	0.41
C+	18 ^{b}	12 ^{b}	-6 ^{a}	15 ^{c}	0.41
M60	12 ^{a}	10 ^{a}	-2 ^{b}	10 ^{a}	0.41
M90	12 ^{a}	11 $^{a b}$	-1 $^{b c}$	11 $^{a b}$	0.41
Mr	13 ^{a}	11 $^{a b}$	-2 ^{b}	12 $^{a b}$	0.41

a , b, and c means within a column, with a common superscript (or with no superscript) are not significantly different from each other (LSD; p< 0.05)

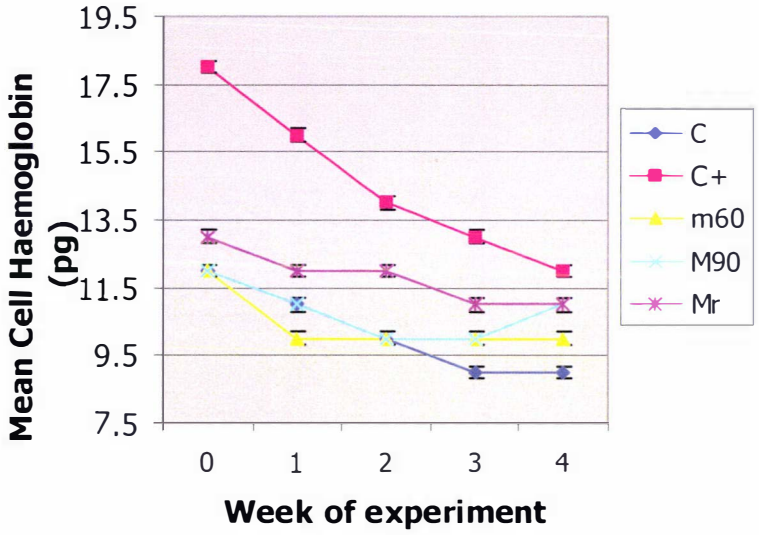


Figure 20: The least-squares means of mean cell haemoglobin (p g) by week for each of the five treatment groups

The data shows that initially the C+ group had mean cell haemoglobin concentration that was significantly different from the other treatment groups. However Subsequent measurements both in the C+ group and the other treatment groups saw a systematic decrease in mean cell haemoglobin concentration. Again this is because piglets are still iron deficient and the red blood cells manufactured are small, and therefore contain smaller amounts of haemoglobin. Decreased mean cell haemoglobin concentration in the C+ groups is a result of the turnover of existing red cells, which are replaced by smaller immature red blood cells. As iron reserves decline as the piglets grow.

5.3.6 Mean corpuscular haem concentration

Statistically significant effects ($P < 0.001$) were observed between the groups and there were also significant differences between pigs within groups, and between weeks. There was no significant interaction between week x diet and between sex x group. The effects of sex were also not significant. The results presented in Table 50 show the least-squares means of the values for mchc obtained from each of the treatment groups.

Table 50: Least-squares means of mchc (g/L)

Group	Week 0	Week 4	Change from week 0 to week 4	Mean value week 0 to week 4	Residual Standard Deviation (RSD)
C	282 [a]	286 [a]	+4 [b]	288 [a]	5.82
C+	309 [b]	299 [b]	-10 [a]	307 [b]	5.82
M60	285 [a]	297 [b]	+12 [c]	291 [a]	5.82
M90	284 [a]	293 [a]	+9 [c]	290 [a]	5.82
Mr	291 [b]	289 [a]	-2 [b]	291 [a]	5.82

a , b, and c means within a column, with a common superscript (or with no superscript) are not significantly different from each other (LSD; p<0.05)

5.3.7 Corpuscular haemoglobin constant

Statistically significant effects (P< 0.001) were observed between the groups and there were also significant differences between pigs within groups, and between weeks. There was also a significant interaction between week x diet. Sex and the interaction between sex x group were not significant. The results presented in Table 51 show the least-squares means of the values for CHCM obtained from each of the treatment groups.

Table 51: Least-squares means of CHCM (g /L)

Group	Week 0	Week 4	Change from week 0 to week 4	Mean value week 0 to week 4	Residual Standard Deviation (RSD)
C	278 [a]	285 [a]	+7 [b]	285 [a]	5.69
C+	308 [c]	296 [b]	-12 [a]	304 [b]	5.69
M60	281 [a]	298 [b c]	+17 [c]	290 [a b]	5.69
M90	280 [a]	294 [b]	+14 [c]	289 [a b]	5.69
Mr	288 [b]	290 [a]	+2 [b]	290 [a b]	5.69

a , b, and c means within a column, with a common superscript (or with no superscript) are not significantly different from each other (LSD; p<0.05)

5.4 Red cell matrix

Table 52 shows the significance of dietary treatments, sex, sex x diet, pig (sex x diet) weeks and week x diet, on red blood cell matrix evaluations. Where the number of red blood cells are categorised by cell haemoglobin concentration and volume and correspond to m1-m9 groups of values within a 3 x 3 matrix.

The r-squared values indicate how much of the variation between the treatments can be explained, by the expanded statistical model shown in section 3.6 (data analysis):

Table 52: The significance of dietary treatment on complete blood count.

	Diet	Sex	Sex x diet	Pig (sex x diet)	Week	Week x diet	R^2
M1	**	ns	ns	***	***	***	0.90
M2	**	ns	ns	***	***	***	0.89
M3	***	ns	ns	***	***	***	0.91
M4	ns	ns	ns	***	ns	***	0.77
M5	**	ns	ns	***	***	***	0.92
M6	*	*	ns	***	***	***	0.80
M7	ns	ns	ns	ns	ns	ns	0.37
M8	ns	ns	ns	***	***	ns	0.68

ns: not significant p>0.05; * p<0.05; ** p< 0.01; *** p<0.001

The results presented in Table 53 show the least-squares means of the values for red blood cells characterised as M1-M9 obtained from each of the treatment groups

Table 53: The least-squares means of red blood cell characteristic M1-M9.

		GROUP					
CELL	WEEK	C	C+	M60	M90	MR	RSD
M1	0	5330 ^{b}	25 ^{a}	8223 ^{c}	5659 ^{b}	3837 ^{b}	1751
	4	13190 ^{b}	4288 ^{a}	4122 ^{a}	3004 ^{a}	2922 ^{a}	1751
M2	0	2543 $^{a\,b}$	244 ^{a}	3280 ^{b}	2528 $^{a\,b}$	1935 $^{a\,b}$	2386
	4	12146 ^{b}	7150 ^{a}	17912 ^{c}	13453 ^{b}	7672 ^{a}	2386
M3	0	43 $^{a\,b}$	4 ^{a}	82 ^{c}	49 ^{b}	34 $^{a\,b}$	31
	4	247 ^{c}	78 ^{a}	211 ^{c}	132 ^{b}	79 ^{a}	31
M4	0	17756 ^{c}	5174 ^{a}	14102 $^{b\,c}$	15216 $^{b\,c}$	11268 ^{b}	3844
	4	11331	11428	13491	15803	15814	3844
M5	0	15916 ^{a}	37480 ^{c}	16224 ^{a}	16418 ^{a}	22341 ^{b}	3687
	4	10570 ^{a}	29021 ^{b}	27703 ^{b}	28951 ^{b}	31684 ^{b}	3687
M6	0	60 $^{a\,b}$	43 ^{a}	97 ^{b}	80 $^{a\,b}$	74 $^{a\,b}$	37
	4	163 ^{b}	126 ^{b}	61 ^{a}	40 ^{a}	40 ^{a}	37
M7	0	51 ^{a}	199 ^{b}	12 ^{a}	39 ^{a}	21 ^{a}	82
	4	-1	6	7	5	15	82
M8	0	186 ^{a}	1142 ^{b}	164 ^{a}	218 ^{a}	775 ^{b}	315
	4	-16	75	-6	-1	-11	315
M9	0	No cells were identified in this category					
	4						

a, b, and c means within a column, with a common superscript (or with no superscript) are not significantly different from each other (LSD; p<0.05)

5.4.1 M1

Statistically significant effects (P< 0.001) were observed between the groups and there were also significant differences between pigs within groups, and between weeks. There was also a significant interaction between week x diet. Sex, and the interaction between sex x diet were not significant. The results presented in Table 53 show the least-squares means of the values for m1 obtained from each of the treatment groups.

5.4.2 M2

Statistically significant effects (P< 0.001) were observed between the groups and there were also significant differences between pigs (sex x group), and between weeks. There was also a significant interaction between week x group. Sex, and the interaction between sex x group were not significant. The results presented in Table 53 show the least squares means of the values for m2 obtained from each of the treatment groups.

5.4.3 M3

Statistically significant effects (P< 0.001) were observed between the groups and there were also significant differences in pig (sex x group), and between weeks. There was also a significant interaction between week x group. Sex, and the interaction between sex x group were not significant. The results presented in Table 53 show the least squares means of the values for m3 obtained from each of the treatment groups.

5.4.4 M4

Statistically significant effects (P< 0.001) were observed between pigs (sex x group) and there was also a significant interaction between week x group. Sex, group and the interaction between sex x group were not significant. The results presented in Table 53 show the least squares means of the values for m4 obtained from each of the treatment groups.

5.4.5 M5

Statistically significant effects (P< 0.001) were observed between the groups and there were also significant differences between pigs (sex x group), and between weeks. There was also a significant interaction between week x group. Sex, and the interaction between sex x group were

not significant. The results presented in Table 53 show the least squares means of the values for m5 obtained from each of the treatment groups.

5.4.6 M6

Statistically significant effects (P< 0.001) were observed between the groups and there were also significant differences between sex, pigs (sex x group), and between weeks. There was also a significant interaction between week x group. Sex, and the interaction between sex x group were not significant. The results presented in Table 53 show the least squares means of the values for m6 obtained from each of the treatment groups.

5.4.7 M7

There were no statistically significant interactions for m7. The results presented in Table 53 show the least squares means of the values for m6 obtained from each of the treatment groups.

5.4.8 M8

Statistically significant effects (P< 0.001) were observed between pigs (sex x group) and between weeks. Group and sex, and the interactions between sex x group and week x group were not significant. The results presented in Table 53 show the least squares means of the values for m8 obtained from each of the treatment groups.

5.4.9 M9

There were no cells identified in this category m9.

The 3 x 3 matrix described the changes in haemoglobin concentration and cell volume over the experimental period. Initially the C+ group had a greater proportion of red cells placed in the "ideal" m5 category. This reflected, that having received 200 mg of supplementary iron shortly after birth that the piglets were not iron deficient and that the bone marrow had sufficient iron to manufacture red cells.

The positive iron status would have inhibited iron absorption from the small intestine, where as the negative iron status in the other treatment groups would have enhanced the absorption of dietary iron. Therefore the dimensions of the red cells changed over the experimental period. C+ and C groups saw an increase number of red cells in the lower matrix categories. The iron reserves in C+ group would have become depleted as a change in physiological state increased the demand for iron. The negative iron status in the C group was compounded by the consumption of dietary non-haem iron, that has a lower bioavailability than haem iron that was consumed by animals in the M60, m90 and mr groups. Therefore insufficient quantities of iron are reaching the bone marrow; as a result red cells with smaller amounts of haemoglobin are being released in to the circulatory system. Like the C group initially the M60, m90 and mr groups have large number of cells in lower matrix categories. But as these animals were consuming diets containing iron that was more bioavailable, the measurements taken at the end of the study show some movement. The M60, m90 and mr groups have increased red cell numbers in the ideal categories and reduced them in others.
The short duration of this study compared with the life span of a red blood cell means that although changes are beginning to emerge it is not possible to see the whole picture as only a small proportion (23%) of the initial red cells have been turned over.

Figures 21 and 22 show the red blood cell counts classified using the 3 x 3 matrix, as a percentage of the total number of red cells.

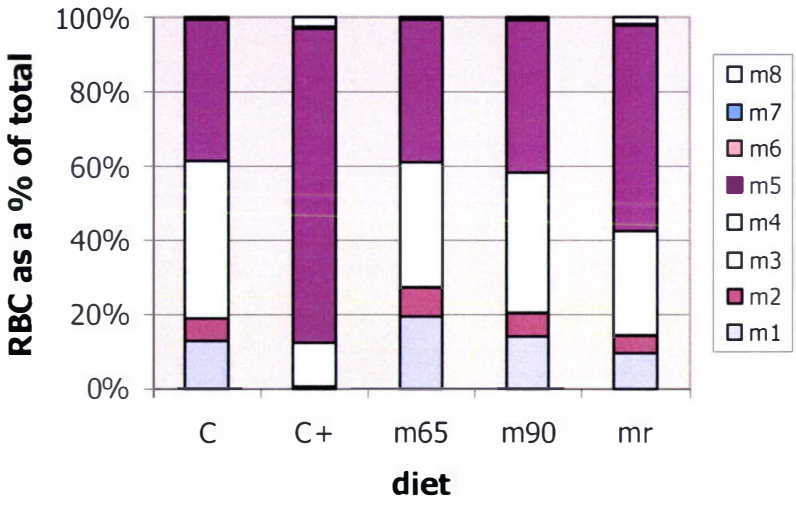


Figure 21: The least squares means of red blood cell characteristic as a percentage of total at the beginning of the study.

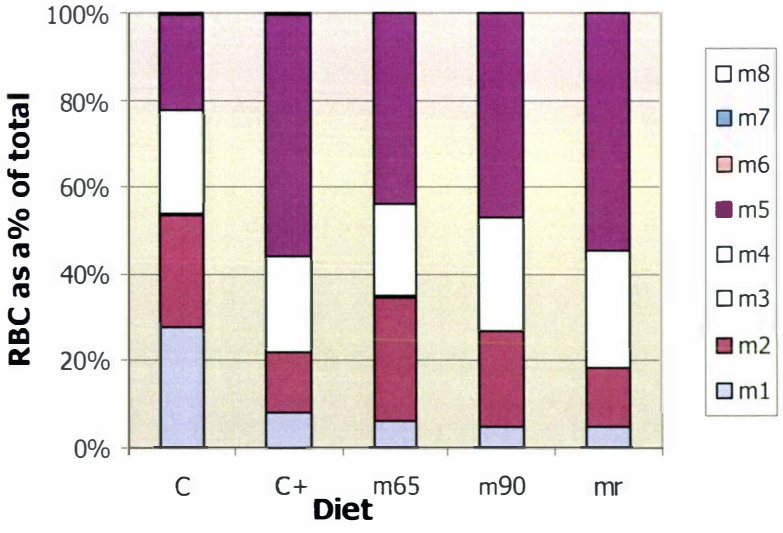


Figure 22: The least squares means of red blood cell characteristic as a percentage of total at the end of the study.

5.5 Serum Iron Concentration

Table 54 shows the significance of the effects of dietary treatments, pigs within diets, weeks and week x diet on serum iron concentration and body haemoglobin iron. The variable sex, sex x diet, and pig (sex x diet) were also tested and were not significant. The statistical model was then re-run without these variables. . The r-squared values indicate how much of the variation between the treatments can be explained, by the statistical model shown in section 3.6

Table 54: Significance levels of dietary treatment on serum iron.

	Group	Sex	Sex x group	Pig (sex x group)	Week	Week x group	R^2
Iron	*	ns	ns	***	***	***	0.64
HGB Fe	**	ns	ns	***	***	***	0.93

ns: not significant p>0.05; * p<0.05; ** p< 0.01; *** p<0.001

Statistically significant effects (P< 0.001) were observed between the groups and there were also significant differences between pigs within groups, and between weeks. There was also a significant interaction between week x group. Sex and the interaction between sex x group were not significant. The results presented in Table 55 and also in Figure 23 show the least-squares means of the values for serum iron obtained from each of the treatment groups.

Table 55: The least-squares means of serum iron content (μmol / L) for each of the treatment groups.

Group	Week 0	Week 4	Change from week 0 to week 4	Mean value week 0 to week 4	Residual Standard Deviation (RSD)
C	1.96 [a]	1.90	-0.06 [a]	1.97 [a]	1.13
C+	3.34 [b]	3.02	-0.32 [a]	3.56 [b]	1.13
M60	1.24 [a]	2.72	+1.48 [b c]	2.21 [a b]	1.13
M90	1.48 [a]	2.34	+0.86 [a c]	2.03 [a]	1.13
Mr	1.70 [a]	3.30	+1.6 [b c]	2.35 [a b]	1.13

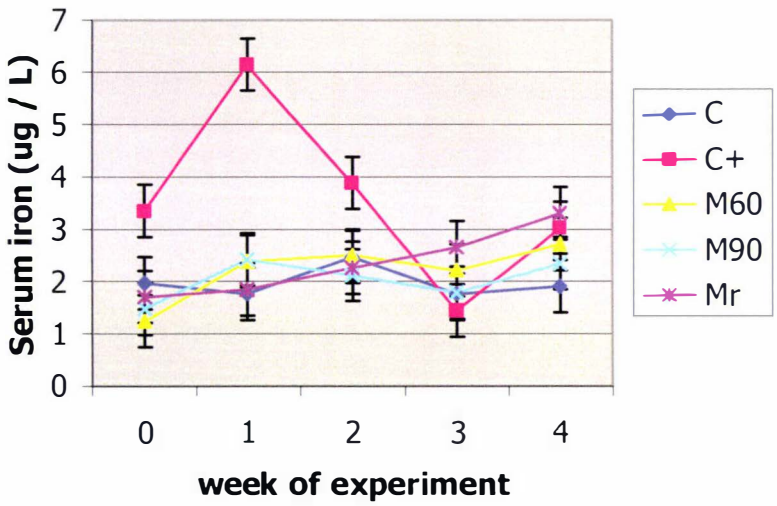
a , b, and c means within a column, with a common superscript (or with no superscript) are not significantly different from each other (LSD; p<0.05)

Initially the C+ group had serum iron levels that were significantly different from the other treatment groups this was anticipated, and was due to the difference in amounts of iron administered shortly after birth (200 mg vs. 60 mg). This was followed by a rapid increase in serum iron (week 1) followed by an equally sharp decrease (weeks 2 and 3) but having serum

iron levels at the end of the study only marginally different from where they began. This highlights the findings of Worwood (1997) who reported that measures of serum iron only provide a snapshot of the iron status of a subject at the specific time when the blood sample is taken. And also that of Furugouri (1971) who demonstrated a day-to-day physiological variation in serum iron and TIBC capacity in pigs.

The other groups began the study with significantly lower levels of serum iron, but which climbed progressively over the study period. The greatest changes occurred in the piglets consuming diets containing meat iron.

Figure 23: Least- squares means (± S. E.) of serum iron values (µmol / L) for each of the treatment groups.



5.6 Iron retention

Statistically significant effects ($P < 0.001$) were observed between the groups and there were also significant differences between pigs within groups, and between weeks. There was also a significant interaction between week x groups. Sex and the interaction between sex and group were not significant. The results presented in Table 56 and also in Figure 24 show the least squares means of the values for body HGB Fe obtained from each of the treatment groups.

Table 56: The least squares means of body haemoglobin iron content (mg) for each of the treatment groups.

Group	Week 0	Week 4	Change from week 1 to week 4	Mean value week 0 to week 4	Residual Standard Deviation (RSD)
C	104.9 [a]	204.4 [a]	+99.5 [a]	135.7 [a]	25.1
C+	175.7 [b]	304.5 [b]	+128.8 [a]	238.2 [c]	25.1
M60	105.2 [a]	309.8 [b]	+204.6 [c d]	189.9 [b]	25.1
M90	98.5 [a]	288.1 [b]	+189.6 [b d]	173.0 [b]	25.1
Mr	111.9 [a]	284.5 [b]	+172.6 [b]	188.9 [b]	25.1

a, b, and c means within a column, with a common superscript (or with no superscript) are not significantly different from each other (LSD; $p<0.05$)

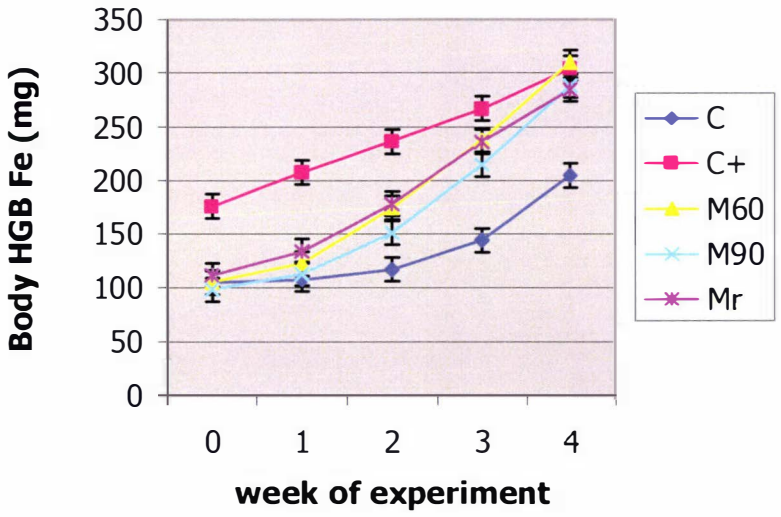


Figure 24: Least-squares means (± S. E) of body Haemoglobin iron values (mg) for each of the treatment groups.

The regression analysis statistics for body HGB Fe x FEI are shown below

	C-	MR	M65	M90	C+	SE
Intercept (a)	96.9[a]	113.8[a]	100.1[a]	93.3[a]	188.0[b]	11.7
Slope (b)	0.163[a]	0.265[bcd]	0.308[bd]	0.254[acd]	0.194[ac]	0.033

a , b, and c means within a column, with a common superscript (or with no superscript) are not significantly different from each other (LSD; $p<0.05$)

Similar to Experiment 1 the diets containing meat iron had better rates of retention than those containing inorganic non-haem iron (C and C+).

The slower initial rate of retention in the C+ group would have resulted from iron status, as they commenced the study with a positive balance of iron in the body, where the other groups had a marginal state of iron deficiency, which would have increased iron absorption from the small intestine. Rapid increases in growth rate accompanied by active erythropoiesis would have been responsible for the continued retention in the C+ group, dietary iron replacing depleting body stores. The M60 group saw body HGB Fe replete more efficiently than either the m90 or mr groups. This suggests that cooking at 60-65ºC was beneficial. Probably by increasing the digestibility of the meat and also the haem iron content of the meat was affected little at this temperature.

5.7 White cells

Table 57 shows the significance of the effects of groups, sex, sex x group, pig (sex x group) weeks and week x group on differential white blood cell counts. The r-squared values indicate how much of the variation between the treatments can be explained, by the statistical model shown in section 3.6
(Where the differential white blood cell counts are those listed in column 1 of Table 57.)

Table 57: Significance levels of dietary treatment on differential white cell count.

	Group	Sex	Sex x group	Pig (sex x group)	Week	Week x group	R^2
White blood cells	ns	ns	ns	***	***	ns	0.57
Neutraphil cells	ns	ns	ns	**	**	ns	0.50
Lymphocyte cells	ns	*	ns	***	***	ns	0.80
Monocyte cells	ns	**	ns	*	**	ns	0.54
Eosinophil cells	ns	ns	ns	ns	***	ns	0.55
Basophil cells	ns	ns	ns	***	***	ns	0.57

ns: not significant p>0.05; * p<0.05; ** p< 0.01; *** p<0.001

Statistically significant effects (P< 0.001) were observed between pigs (sex x groups) and between weeks. Group and sex, and the interactions between sex x group and week x group were not significant. The results presented in Table 58 and also in Figure 25 show the least-squares means of the values for white blood cells obtained from each of the treatment groups.

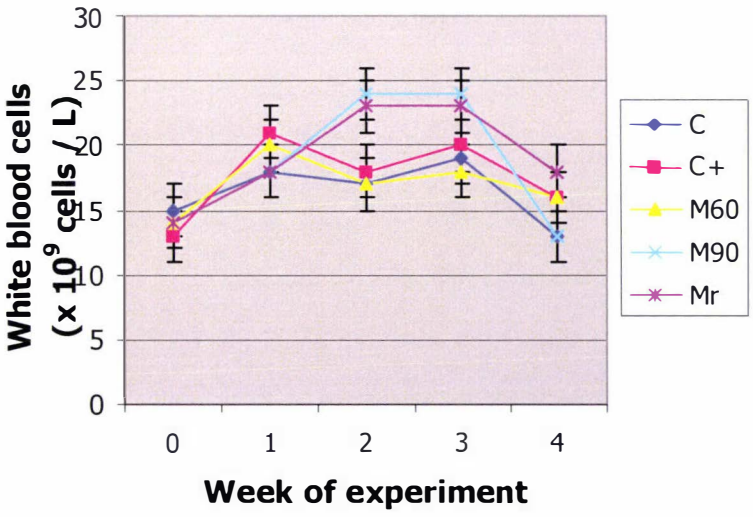
Table 58: Least-squares means of white blood cells (x 10^9 cells / L) for each of the treatment groups.

Group	Week 0	Week 4	Change from week 0 to week 4	Mean value week 0 to week 4	Residual Standard Deviation (RSD)
C	15.4	12.9	-2.5[a]	16.3	4.86
C+	16.4	15.9	- 0.5[a]	18.2	4.86
M60	13.6	16.0	+2.4[a]	17.1	4.86
M90	14.4	13.1	-1.3[a]	18.6	4.86
Mr	13.8	18.2	+4.4[b]	19.1	4.86

a , b, and c means within a column, with a common superscript (or with no superscript) are not significantly different from each other (LSD; p<0.05)

Changes to white blood cells counts were monitored over the experimental period, as the newly weaned piglet is vulnerable to infection. Individual animals reported sporadic increases in white cell count but these increases were not associated with the specific dietary treatments.

Figure 25: The least squares means (± S. E.) of white blood cells (x 10^9 cells / L) by week for each of the five treatment groups.



5.7.1 Neutrophil

Statistically significant effects (P< 0.001) were observed between pigs (sex x group) and between weeks. Group and sex, and the interaction between sex x group and week x group were not significant. The results presented in Table 59 show the least-squares means of the values for neutrophil cells obtained from each of the treatment groups.

Table 59: Least-square means of neutrophil cells (x 10^9 cells / L) for each of the treatment groups.

Group	Week 0	Week 4	Change from week 0 to week 4	Mean value week 0 to week 4	Residual Standard Deviation (RSD)
C	2.5	4.1	1.6 [a]	3.6	3.61
C+	3.2	4.3	1.1 [a]	4.2	3.61
M60	2.9	4.9	2 [a]	4.1	3.61
M90	2.7	2.4	-0.3 [a]	5.2	3.61
Mr	1.7	6.0	+4.3 [b]	5.5	3.61

a , b, and c means within a column, with a common superscript (or with no superscript) are not significantly different from each other (LSD; p<0.05)

5.7. 2 Lymphocyte

Statistically significant effects (P< 0.001) were observed between sex, pigs (sex x group) and weeks. Groups, and the interactions between sex x group and week x group were not significant. The results presented in Table 60 show the least- squares means of the values for lymphocyte cells obtained from each of the treatment groups.

Table 60: Least- squares means of lymphocyte cells (x 10^9 cell / L) for each of the treatment groups.

Group	Week 0	Week 4	Change from week 0 to week 4	Mean value week 0 to week 4	Residual Standard Deviation (RSD)
C	10.9	7.6[a]	-3.3 [a]	10.9	1.8
C+	10.8	10.0 [b]	-0.8 [b]	11.9	1.8
M60	9.2	9.6 [a b]	+0.4 [b]	11.3	1.8
M90	9.6	9.3 [a b]	-0.3 [b]	11.3	1.8
Mr	10.3	10.5 [b]	+0.2 [b]	11.7	1.8

a , b, and c means within a column, with a common superscript (or with no superscript) are not significantly different from each other (LSD; p<0.05)

5.7.3 Monocytes

Statistically significant effects (P< 0.001) were observed between sex, pigs (sex x group) and between weeks. The interactions between sex x group and week x group were not significant. The results presented in Table 61 show the least-square mean of the values for monocyte cells obtained from each of the treatment groups.

Table 61: Least-square means of monocyte cells (x 10^9 cells / L) for each of the treatment groups.

Group	Week 0	Week 4	Change from week 0 to week 4	Mean value week 0 to week 4	Residual Standard Deviation (RSD)
C	1.4	0.4	-1	0.9	0.58
C+	1.6	0.7	-0.9	1.1	0.58
M60	0.9	0.7	-0.2	0.9	0.58
M90	1.6	0.6	-1	1.3	0.58
Mr	1.0	0.8	-0.2	1.1	0.58

a , b, and c means within a column, with a common superscript (or with no superscript) are not significantly different from each other (LSD; p<0.05)

5.7.4 Eosinophils

Statistically significant effects (P< 0.001) were observed between weeks. Group, and sex, and the interactions between sex x group, pig (sex x group) and week x group were not significant. The results presented in Table 62 and also in Figure 68 show the least-square mean of the values for eosinophil cells obtained from each of the treatment groups.

Table 62: Least-square means of eosinophil cells (x 10^9 cells / L) for each of the treatment groups.

Group	Week 0	Week 4	Change from week 0 to week 4	Mean value week 0 to week 4	Residual Standard Deviation (RSD)
C	0.12	0.32	+0.20	0.2	0.19
C+	0.13	0.55	+0.42	0.3	0.19
M60	0.02	0.33	+0.31	0.2	0.19
M90	0.05	0.37	+0.32	0.2	0.19
Mr	0.08	0.42	+0.34	0.2	0.19

a , b, and c means within a column, with a common superscript (or with no superscript) are not significantly different from each other (LSD; p<0.05)

5.7.5 Basophil

Statistically significant effects (P< 0.001) were observed between pigs (sex x group) and between weeks. Group and sex, and the interaction between sex x group and week x group were not significant. The results presented in Table 63 show the least- squares means of the values for basophil cells obtained from each of the treatment groups.

Table 63: Least-squares means of basophil cells (x 10^9 cells / L) for each of the treatment groups.

Group	Week 0	Week 4	Change from week 0 to week 4	Mean value week 0 to week 4	Residual Standard Deviation (RSD)
C	0.55	0.09	-0.46	0.29	0.26
C+	0.39	0.11	-0.28	0.26	0.26
M60	0.26	0.14	-0.12	0.31	0.26
M90	0.43	0.17	-0.26	0.29	0.26
Mr	0.38	0.16	-0.22	0.23	0.26

a , b, and c means within a column, with a common superscript (or with no superscript) are not significantly different from each other (LSD; $p<0.05$)

Chapter 6

6. Discussion

Iron status effects

A general objective of this study was to determine the effect of iron status on iron absorption using both anaemic piglets and piglets with a positive iron status. Piglets are born with limited reserves of iron, which decline rapidly over the first few weeks of life. Anaemia is prevented in the farmed pig by the administration of supplementary iron shortly after birth.
Typically the red cell mass contains about 80% of all absorbed iron, (Finch and Huebers 1991) where it is present as haem, a substituted porphyrin with a central iron atom. Haem is used in the formation of haemoglobin, the protein found in red blood cells, which is used to transport oxygen, H^+ ions and carbon dioxide to and from body tissues.
At the beginning of experiment 1, the physiological difference in iron status had already manifested itself in behavioural differences between the groups of piglets. The anaemic piglets in C, Cv250, Cv500 and meat groups were lethargic and displayed inappetence. The non-anaemic piglets in the C+ group were generally livelier.

During the initial days of the study (days 0-6) feed intake was low irrespective of treatment group. The low intake resulted in negative rates of growth in the groups containing iron deficient animals; only piglets in the C+ group recorded small positive increases in liveweight.
Poor intake and the subsequent loss of weight and body condition are not uncommon in the immediate post-weaning period. English et al. (1984) reported several studies that found that low feed intake and loss of body weight and condition at weaning could be as a result of age and weight at weaning, and also the change in environment and the transition from milk to a novelty diet that takes place as a result of weaning.
Days 7-13 of the experiment saw the continuation of poor feed intake. This was despite the addition of a raspberry flavour to the diet. However, Wise (1991) found that animals suffering from a nutrient deficiency might reduce their food intake. This may provide a more probable explanation, as the C+ group that had been injected with iron shortly after birth, and were therefore not iron deficient, had significantly higher intakes than those of the other treatment groups throughout the study period.
The diets used in this study were unlabelled; therefore the determination of iron bioavailability was made from repeated measurements of some blood parameters and the calculation of iron

retention. The blood parameters were those listed in Table 14, and the calculation of iron retention shown in section 3.6.
Changes to the blood parameters, which make up the red cell mass, were generally in line with cumulative feed intake. Therefore until days 14-27 of the experiment, when feed intake increased considerably, there was little variation in the composition of the red cell mass.
Unfortunately the poor feed intake prolonged the period of time that the piglets were iron deficient creating a no-win situation. As the iron deficiency, induced inappetence, which would continue until piglets obtained essential iron from the diet.

As feed intake increased, the effect of iron status on the absorption of iron became more evident. Animals with a positive iron status (C+ group) had relatively stable blood parameters. This was because the blood parameters were already within normal physiological ranges for healthy pigs; also there was sufficient iron available within the piglets' bodies that enabled blood parameters to be maintained within the normal physiological range throughout the 28-day study.
The anaemic piglets saw increases in the blood parameters occur only when feed consumption increased. This demonstrates that in these animals iron stores were deplete, and therefore iron was unavailable to be used in the manufacture of red blood cells.
The changes to blood parameters were also comparative to feed intake. Piglets consuming the meat diet had significantly higher feed intakes than those of the C, CV250, and CV500 groups; therefore iron intake in these animals was also higher. This resulted in greater changes in the blood parameters, also the rate at which the increases occurred were more rapid than those of the other anaemic piglets. This demonstrates that in iron-deficient piglets, dietary iron was actively absorbed from the gastrointestinal tract, where as in non-iron deficient piglets, the active absorption of dietary iron was not evident.
Measures of iron retention were calculated using cumulative iron intake (feed intake x iron content of the diet) correcting for any differences that may have occurred as a result of variation in feed intake. The accumulation and retention of body haemoglobin iron increased in all groups from day 14 of the study. Of the iron deficient piglets, the meat group had significantly higher retention rates than the C, CV250 or CV500 groups, indicating that there are differences in bioavailability between meat and non-meat iron. Meat iron is more bioavailable than non-meat iron. Iron retention also increased in the C+ group, in spite of the positive iron status. This is because the piglets are growing, the changes to the piglets physiological state would see stored iron used to manufacture additional red blood cells, therefore body haemoglobin iron is increased.

Iron bioavailability for piglets

In addition to changes in the way iron is absorbed in iron deficient piglets. The diets containing either meat iron or non–meat iron in the vegetable based diet began to retum piglets to haematologic normality at different rates.

By day 21 of the study there were increases in a number of the blood parameters in the Cv250, Cv500 and C groups but the increases in blood parameters were most apparent in the meat group. There were significant differences between the groups consuming meat iron and those consuming non-meat iron in blood haemoglobin concentration, mean cell volume and mean cell haemoglobin.

This pattern was not however repeated in the evaluation of mean corpuscular haem concentration or in corpuscular haemoglobin constant. There were no significant differences between groups consuming either meat iron or non-meta iron in measurements of these two parameters.

Additionally red blood cell counts, in piglets in the meat group were not significantly different to those of piglets consuming the Cv500 diet, but were significant from the C and Cv250 groups. Increased feed intake at this time would have resulted in the dietary iron being present in the gastrointestinal tract. The negative iron status would see iron actively absorbed and incorporated into blood products containing iron. As other variables have been excluded, differences in the rate of repletion could be attributed to the difference in bioavailability between the meat iron and the inorganic non-haem iron found in the vegetable based diet.

This would be consistent with the findings of South et al. (2000) who established that in pigs' meat iron is more readily absorbed (15-35%) than non-haem sources of iron (2-20%). Bjorn – Rasmussen et al. (1974) proposed that the differences in bioavailability were because dietary iron forms two pools within the intestinal lumen, a haem pool and a non-haem iron pool. Also the two sources of iron are absorbed using different pathways (Conrad et al. 1999). The meat iron consisting mostly of organic haem iron is more readily assimilated as it taken up directly by the enterocyte cells of the small intestine as an intact porphyrin. Whereas the inorganic non-haem iron has to be solubilised before it can be absorbed. These pathways are described in section 2.3.

The effects of vitamin C: Iron bioavailability in iron deficient piglets consuming non-haem iron sources, supplemented with vitamin C.

In addition to differences in the bioavailability of meat iron and non-meat sources of iron, a further general objective of the study was to determine the effects of vitamin C on the absorption of non-meat iron. The study established that, of the three groups consuming diets containing

non-meat iron in vegetable based diets; there were significant differences in some blood parameters resulting from the addition of Vitamin C.

Vitamin C has been shown to enhance the bioavailability of non-haem iron in iron deficient subjects, consuming either semi-synthetic or experimental diets. This is shown in Table 2. The role of vitamin C in enhancing non-haem iron from a complete diet is less clear. This study used food choices from a typical human diet and found that overall there were no significant differences between number of red blood cells and haematocrit between the C and CV250 diet, containing 0, and 250 ppm of vitamin C, yet the numbers observed in the CV500 were significantly different from both the C and CV250 diets. This pattern was not however repeated in the evaluation of mean cell volume, mean cell haemoglobin, mean corpuscular haem concentration or in corpuscular haemoglobin constant. The blood haemoglobin concentration in piglets consuming the CV500 diet was significantly higher than for the C diet during week four of the study but not overall.

This suggests that the vitamin C may be utilized in the transfer of iron so that it can be used in the production of red blood cells rather than increasing bioavailability in the small intestine. In which case the vitamin C has to be included in the diet at high levels. Under these experimental conditions 500 ppm (as found in the CV500 diet) appears to be the most beneficial.

Red blood cells are produced in the bone marrow and pass through several stages of development before being released in to the circulatory system (as shown in Appendix 5). In addition to the number per volume of blood, red blood cells in circulation can also be characterised, by volume (V) and haemoglobin concentration (HC) within the cells, using a 3 x 3 matrix using the abbreviations m1-m9.

A blood sample taken from a healthy non-iron deficient piglet would have blood cells with a HC reading between 110-170 g / L with a corresponding V measurement of 50-68 fL corresponding to the m5 position. Values below these parameters (m1-m4) indicate that small immature reticulocytes were prematurely entering the circulatory system, where as values above these levels (m6-m9) suggest that cells are larger, as protoporphyrin accumulates in the cells.

A change in categorisation of the red blood cells was not evident as a result of feeding the control diet supplemented with 0, 250 or 500 ppm of vitamin C. There were no significant difference between the numbers of cells placed in categories m1, and m3-5. The Cv500 group had a greater number of cells placed in the m2 and m6-m9 categories than either the C or Cv250 group, characteristic of prolonged iron deficiency. The increase in the number of red cells in the matrix categories (m6-m9) suggests that high levels of vitamin C may play a role in the accumulation of protoporphyrin in cells in the absence of sufficient iron.

Measurements of body haemoglobin iron also show that in iron deficient piglets, consuming non-meat iron supplemented with vitamin C, iron accumulates more slowly than in those consuming meat iron. Additionally there were no significant differences between the various levels of vitamin C and the rate at which iron accumulated in the red cell mass.

Iron bioavailability in non-iron deficient piglets

Having received an iron injection shortly after birth, piglets in the C+ group were not iron deficient and an evaluation of the red blood cells from these animals showed that approximately 90% of red cells were classified in the optimum m5 position. There was little variation in the numbers of red cells in this category throughout the study showing that in these piglets there were significant quantities of iron available in the body to sustain active erythropoiesis.
This was supported by measurements of serum iron concentration, and unsaturated iron binding capacity. There were small deviations in the serum iron concentration measurements taken throughout the study; Worwood (1997) reported that this was because serum iron pools are turned over so rapidly (10-20 times per day). However, used in conjunction with measurements of unsaturated binding capacity, it can be determined that there was little variation in the proteins ability to bind iron. This provided further evidence that in non-iron deficient piglets iron was not being actively absorbed.

The effectiveness of meat and non-meat sources of iron in returning iron deficient piglets to haematologic normality.

At the beginning of the study the extent of the iron deficiency, and its role in the production of healthy red blood cells is already evident, as all iron deficient piglets in the C, Cv250, Cv500 and the meat groups had larger numbers of red blood cells in the lower matrices category. This is consistent with the findings of Pippard (1995), who reported that iron deficiency impaired haemoglobin synthesis and as a result the red blood cells tended to be small and poorly haemoglobinised. However as the natural life of red blood cells is approximately 120 days, the 28-day study would see only 23% of the initial red blood cells turned over. Therefore only small changes in classification would be evident. Nevertheless, by week 4 of the study there were significant differences between piglets consuming meat and non-meat iron, and the placement of red blood cells within the matrix. The meat group had increased the number of red blood cells in the optimum m5 category. In contrast piglets consuming non-meat sources of iron (C, Cv250 and Cv500 groups) had decreased numbers of red cells in this position. This supports the earlier findings of this study, in that meat iron is more bioavailable than non-meat sources of iron, resulting in a more rapid return of iron-deficient piglets to haematologic normality over those

consuming non-meat sources of iron. Measurements of body haemoglobin iron retention also show the same difference in the effectiveness of meat iron and non-meat iron in returning iron deficient piglets to haematologic normality. The body haemoglobin iron accumulated more rapidly in the iron deficient piglets consuming the meat iron than those consuming the inorganic non-meat iron alternative source of iron.

Experiment 2

The effects of cooking temperature on iron bioavailability in meat.

The general objective of experiment 2 was to determine the effects of cooking temperature on iron bioavailability in meat, using 100% visual lean bull beef that remained uncooked (mr) or that had been subject to cooking in thermostatically controlled circulating water baths at either 60ºC (m60) and 90ºC (m90) and incorporated into the diet at 25% of the total dietary ingredients (on an as-fed basis).

As in experiment 1, measures of bioavailability were made by measuring the rate of repletion of some of the elements of the red cell mass and by the calculation of iron retention.

Piglets in the m60 and m90 groups consistently had significantly better rates of repletion than the mr group, in number of red blood cells per volume of blood, level of haematocrit, blood haemoglobin concentration, mean corpuscular haemoglobin concentration and corpuscular haemoglobin constant. This is in agreement with Newsome (1980) who reported that the net effect of heat processing was positive, by increasing the nutrient and / or digestibility of food components. In this case the cooking process would have initiated the denaturation of some of the meat proteins; enabling the porphyrin complex, containing the iron to be released. Whereas piglets consuming the mr diet containing the raw uncooked meat protein would rely solely upon enzymatic hydrolysis that takes place within the digestive tract to release the iron from the meat.

The lack of clarity between the m60 and m90 groups, despite differences in cooking temperature could be because meat contains both haem and non-haem fractions of iron. Purchas et al. (2001 unpublished data) calculated that in uncooked meat these values were 85% and 15% respectively, for haem and non-haem iron.

Kristensen and Purslow (2001), Schricker and Miller (1983) and Purchas et al. (2001 unpublished data) have demonstrated that heat processing of meat produced an incremental decrease in haem iron content as a function of heating temperature. So that the haem iron content of meat begins to decrease when processing temperatures exceeds 55ºC. This is because processing heat affects the thermal stability of the muscle myoglobin, causing denaturation, which releases and causes degradation to the haem molecule (Kristensen and Purslow 2001).

The meat used in the m60 diets was subject to relatively mild heat processing that would have initiated the denaturation of some of the meat proteins, facilitating the release of the porphyrin complex. However, the heat treatment (60ºC) may not have been harsh enough to see a significant reduction in the haem iron content (Schricker and Miller 1983).

In contrast, the m90 diet containing meat that had been subjected to a much higher cooking temperature would have seen a significant decrease in the haem iron content as it was converted into non-haem iron. Therefore, the total iron in the m90 diet would be the same as that of the

m60 diet, but as a result of cooking, a greater proportion of the iron would be non-haem iron (78.25% vs. 68.6%).
Data from experiment one showed that non-meat iron has a lower bioavailability than that of meat iron. Although the absorption of non-meat iron can be enhanced by specific amino acids found in meat as outlined in section 2.7. It may be that the presence of these amino acids increased the bioavailability of the non-haem iron fraction so that overall, iron bioavailability between the m60 and m90 group were not significantly different.

Initially serum iron levels in the mr, m60 and m90 groups reflected difference in iron administered shortly after birth (60 mg vs. 200 mg administered to the C+ group). The levels of serum iron increased as the study progressed so that by week 4 of the study serum iron concentrations in the mr group equalled that of piglets in the C+ group. This shows clearly the effectiveness of meat iron in returning anaemic piglets to haematologic normality, despite an initial 140 mg iron deficit. Diets containing cooked meat also had changes in serum iron concentration that were significantly higher than piglets consuming non-meat iron.

Similar to experiment one, the accumulation and retention of body haemoglobin iron increased in all groups throughout the study. Of the iron deficient piglets, the groups consuming meat diets had significantly higher retention rates than the C group; indicating the difference in bioavailability between meat and non-meat iron. Of the groups consuming the meat diets, retention rates were highest in the m60 group. This indicates that cooking at 60ºC was beneficial. Probably by facilitating the release of the porphyrin complex, as the muscle proteins were denatured and additionally the haem iron content of the meat would be least affected at this temperature.
Iron retention also increased in the C+ group, but remained below that of the groups consuming meat. Again increases in iron retention in piglets that initially had a positive iron status is because the piglets are growing rapidly, the changes to the piglets physiological state would see stored iron used to manufacture additional red blood cells, therefore body haemoglobin iron is increased. However, blood haemoglobin concentrations of piglets in the C+ group declined from week one of the study indicating that there was insufficient stored iron to meet the piglets' requirements. Also animals in this group are also consuming a diet containing non-meat iron that has lower bioavailability than that of meat iron. So that overall the accumulation and retention rates of these piglets were lower than that of the m60, m90 and mr groups.

The effectiveness of meat iron in bringing about a return of anaemic piglets to haematologic normality.

The 3 x 3 matrix described the changes in haemoglobin concentration and cell volume of the red blood cells over the experimental period.

To prevent the lethargy displayed by the iron deficient piglets in experiment 1, in addition to the 200 mg of supplementary iron administered shortly after birth to piglets in the C+ group, 60 mg of supplementary iron was administered to piglets in the C, m60, m90 and mr groups providing a marginal state of iron deficiency.

This was evident in the placement of cells within the matrix. Initially piglets in the m60, m90 and mr groups had larger number of cells in the lower matrix categories. This is symptomatic of the red cell mass containing large numbers of small immature red blood cells, but is consistent with Pippard (1995) who reported that iron deficiency impaired haemoglobin synthesis and as a result the red blood cells tended to be small and poorly haemoglobinised. But unlike experiment 1, the small amount of supplementary iron administered to the m60, m90 and mr groups shortly after birth-prevented inappetence. Consequently feed intake was much higher than in experiment 1, averaging 552 ± 75g / day at the beginning of the study and increasing to 1147 ± 33 g / day at the end of the study. So despite the relatively small turnover of initial red blood cells, at the end of the study there was an improvement in the placement of cells within the matrix.

Piglets consuming the diets containing meat increase the number of cells placed in the optimum m5 category, whereas the piglets in the C and C+ group saw a reduction in the number of cells placed here. The increased number of cells placed in the optimum category show that the meat iron was readily assimilated, maintaining active erythropoiesis when body weight was increasing.

Iron bioavailability in anaemic and non-anaemic piglets consuming non-meat iron.

Again using the matrix to analyse the volume and haemoglobin concentration of red blood cells. Initially the C+ group had a greater proportion of red cells placed in the "ideal" m5 category. This reflected, that having received 200 mg of supplementary iron shortly after birth that the piglets were not iron deficient and that the bone marrow had sufficient iron to manufacture red cells. The positive iron status of the piglets in this group would have inhibited further iron absorption from the small intestine until iron reserves were depleted.

Piglets used in Experiment 2 saw growth rates increase by 266 ± 47 g / piglet/day during the 28-day study period, therefore despite the administration of supplementary iron, piglets in the C+ group had insufficient stores of iron to main active erythropoiesis during changes in physiological state, which would have increased the demand for iron. This is seen in the fluctuating serum iron levels; the change coincides with changes in cellular demand for iron as the animal grows (Furugouri and Kawabata 1976). Changes in the regulatory processes that would have enabled

the piglets to increase iron absorbed from the gastrointestinal tract take place over time and therefore are not apparent.
In contrast, the negative iron status of piglets in the C group would have seen iron actively been absorbed from the small intestine. Unfortunately piglets in this group were fed a diet containing non-meat iron, which has a lower availability than that of meat iron (South et al. 2000).
Therefore iron was not available in sufficient quantities to support the active erythropoiesis taking place during the physiological state of growth. Therefore red blood cells produced are in the lower matrix categories and cell turnover would decrease the number of cells placed in the optimal position.

Body haemoglobin iron in these two groups increased, yet at a slower rate of repletion than that of the mr, m60 and m90 groups. In the C+ group the lower rate of repletion would be due to iron status, as they commenced the study with a positive balance of iron in the body whereas the other groups had a marginal state of iron deficiency. The lower rate of depletion in the C group than that of other anaemic piglets resulted from the difference in bioavailability of iron. Meat iron whether raw or cooked is more bioavailable than non-meat iron.

The m60, m90 and mr groups began the study with marginal levels of iron deficiency having received only 60 mg supplementary iron shortly after birth. These groups saw blood haemoglobin concentrations incrementally increase throughout the study. The animals in two of these groups the m60 and m90, finished the study with haemoglobin concentrations statistically higher than the C+ group. This occurred despite the initial 140 mg iron deficit.
Of the 3 groups consuming haem iron, the increases in blood haemoglobin concentration were greatest in the m60 and m90 groups.
An assumption can be made that not all food processing is necessarily detrimental to the nutritive value of food as the blood haemoglobin concentrations were higher in the groups consuming processed meat than that of the mr group that consumed raw unprocessed meat.
The differences between the processed and unprocessed meat are in general agreement with Han et al (1993) who measured the haem iron content of beef as a function of heating temperature and found that an inverse relationship exists between haem and non-haem iron content of the meat.
In this case the heat treatment would have caused changes to the ionisation states of the amino acids causing the protein to denature, facilitating the release and degradation of the iron containing porphyrin complex. At the same time the non-haem iron content would have increased due to the conversion of haem iron into non-haem.

Concluding discussion of experiments 1 and 2

Repeated hematologic measurements were used in this series of experiments to identify the rate of repletion in several blood parameters following the consumption of meat, vegetable and milk based diets over a 28-day period. Table 64 shows a summary of the changes over the 4-week period in the blood parameters between the treatment groups in experiment 1.

Table 64: A comparison of changes over the 4-week period in blood parameters for experiment 1.

Blood parameter	Control	Control + 250 ppm Vit C	Control + 500 ppm Vit C	Meat
Red blood Cells ($x\ 10^{12}$ cells/ L)	+1.37	+1.22	+1.93	+1.95
Haemoglobin (g / L)	+4.4	+4.0	+10.4	+22.0
Haematocrit (L / L)	+0.02	+0.02	+0.05	+0.09
Mean Cell Volume (fL)	-7	-7	-5	+2
Mean Cell Haemoglobin (p g)	-2	-2	-2	0

A direct comparison of the 4 treatment groups that had equivalent iron status shows that at the end of the trial piglets consuming the meat diet had higher numbers of red cells, blood haemoglobin concentration, haematocrit, mean cell volume, and mean cell haemoglobin than those consuming vegetable based diets. As the diets were comparable in digestible energy (DE), DE: lysine ratio and the piglets in these four groups had equivalent iron status, the results indicate that the differences in the blood parameters were a result of the two dietary forms of iron being consumed by these groups. The haem iron found mainly in meat was significantly more bioavailble than the non-meat iron found in the vegetable based diets. This is consistent with the findings of South et al (2000) who also observed differences in bioavailability when pigs were fed meat and non-meat sources of iron..

In addition to the quantitative difference in the blood parameters the degree to which the haematologic parameters changed were also of interest. The meat group had a higher rate of repletion than those consuming the vegetable diets containing non-haem iron. However, of the animals consuming non-haem iron the groups consuming non-haem iron that had been fortified with vitamin C at 500-ppm (CV500) had higher rates of repletion than those consuming either 250-ppm of vitamin C (Cv250 group) or the C group consuming solely non-haem iron. The differences in the degree of repletion point to the differences in the way the two forms of iron are pooled and then transported in to the body using the haem uptake pathway and the DCT-1 pathway for haem and non-haem iron, respectively.

Haem iron is transported directly into the enterocyte using the haem uptake pathway. This is in contrast to non-haem iron that initially has to be reduced and maintained in its ferrous form prior to absorption and also has to overcome problems of competitive inhibition, as the transporters are shared with other divalent ions (as shown in section 2.3).
Therefore the results are indicative of the reduction potential of vitamin C, however a high level (500 ppm) is necessary to maintain a pH in the small intestine that would enable the non-haem iron to be reduced to a more soluble ferrous form.
This is consistent with the findings of Clydesdale (1982) who found that bioavailability of non-haem iron is directly correlated to acid solubility; the acidity increasing ionisation and favouring the ferrous form which has a greater solubility at the pH of the intestine.
The results presented also establish the basis of the intrinsic relationship between iron status and iron absorption. A comparison of the C and C+ group, which differ solely in iron status show that in the iron deficient subject iron is actively absorbed.
In comparison hematologic parameters in the C+ group that showed less movement, indicative of number of red cells and blood haemoglobin concentrations that are appropriate for the age of the animal and have not been impaired by iron deficiency.

The hypothesis of experiment 2 built on the findings from the initial experiment; that meat iron is more bioavailble than non-meat iron. Experiment 1 used raw meat; the 2nd experiment therefore took the next logical step, the effect of processing heat or cooking on the bioavailability of haem iron.
Again using a repletion study, the rates of repletion to a number of blood parameters were measured over a 28-day study period.
Table 65 shows a comparison of the changes over the 4-week study period in the blood parameters between the treatment groups in experiment 2

Table 64: A comparison of changes over the 4-week study period in blood parameters experiment 2.

Blood parameter	Control	Raw Meat	Meat cooked at 60°C	Meat cooked at 90°C
Red blood Cells ($x\ 10^{12}$ cells/ L)	+1.4	+2.5	+3.5	+3.4
Haemoglobin (g / L)	-1	+18	+29	+30
Haematocrit (L / L)	0	+0.1	+0.1	+0.1
Mean Cell Volume (fL)	-11	-7	-7	-7
Mean Cell Haemoglobin (p g)	-3	-2	-2	-1

The number of red cells, blood haemoglobin concentration, level of hematocrit, mean cell volume and mean cell haemoglobin were highest in the piglets consuming heat processed meat.
Although there was a distinction between raw and heat processed meat diets, there was little difference between the levels of repletion of the blood parameters between meat cooked at 60 °C and 90°C as contained in the M60 and M90 diets respectively.
The results show that the difference in repletion rate between raw and cooked meat could be attributed to digestibility. The cooking process causes some of the meat proteins to denature, which may facilitate the release of porphyrin complex, which may increase its digestibility. This is Consistent with Newsome (1980) who reported that the net effect of food processing was positive; increasing the nutrient content and/ or digestibility of the food component.
The lack of clarity between the two heat-processed diets is more difficult to explain. Work by Kristensen and Purslow (2001) and Hans et al. (1993) both show that heat processing in pork, chicken and beef decreases the haem iron content of the meat.
The m60 diet was heat processed to 60° C; this coincides with the temperature necessary to denature the myoglobin molecule. Meat exposed to processing temperatures in excess of 80° see further reductions in haem iron content due to the degradation of the porphyrin complex.
Therefore the expectation was that the animals consuming the m60 diet would have better rates of repletion than those consuming the m90 diet.
Unexpectedly an inverse relationship exists between haem and non-haem iron, as a function of heating temperature (Han et al 1993). Therefore as processing heat increases some of the haem iron is converted to non-haem iron, thereby increasing the non-haem iron content.
Therefore it would seem likely that the m90 diet containing beef processed to 90°C, would have decreased its haem iron content, but the non-haem iron content of the meat would have actually increased. Non-haem iron has lower bioavailability than haem iron, but its bioavailability can be enhanced by some amino acids found in meat. Therefore an assumption can be made that the bioavailability of converted non-haem iron has been increased in a similar way in which vitamin C

enhanced the absorption of non-haem iron in experiment 1. The m60 diet contained meat heat processed to 60°C high enough to denature the myoglobin molecule but not harsh enough to significantly reduce the haem iron content of the meat. Therefore the overall iron bioavailability could be alike. Thereby providing a possible solution to the similar rates of repletion in the animals consuming the m60 and m90 diets.

One of the aims of this study was to validate the piglet as a model that could be used to evaluate iron bioavailability. Although direct comparisons of existing studies in iron bioavailability between piglets and humans are difficult to carry out as they are measured using different haematologic parameters. A review of several experiments that investigated the relationship between diet type and iron bioavailability showed that Massey University research results (this study) using the piglet as an animal model reflected the findings of the human studies therefore providing further evidence that the piglet is a good model for future human iron bioavailability studies.
Table 65 shows a summary of the findings.

Table 65: A comparison of changes in blood parameters in the human and piglet model.

Diet	Species	HGB	RBC	Serum Fe	Repletion	Absorption	References
Milk	Pig	↑	↑	=	↑	↑	Massey University unpubl.
	Human	↑					Hertrampf et al. (1998)
Vit C	Pig	↑	↑	=			Massey University (this study)
	Human			↑		↑	Cook et al (2001)
Cooking	Pig	↑	↑	=	↑		Massey University (this study)
	Human			↑			Martinez et al (1998)
Haem	Pig	↑	↑	=	↑		Massey University (this study)
	Human	↑					Roughead and Hunt (2000)

Chapter 7

7. The utilisation of findings.

The aim of this study was to evaluate a model, using piglets that could be used to evaluate the bioavailability of iron from the whole diet for humans.

Bioavailability is difficult to evaluate, as it is not an absolute value. Iron is extremely sensitive to the pH changes that occur along the digestive tract. Additionally iron can form complicated associations with other dietary nutrients that can change the way in which it is assimilated by the body.

Iron deficient piglets were used as human models in a repletion study, which evaluated the rate of regeneration of a number of blood parameters. Following the consumption of diets containing ingredients typical of the human diet.

The results show that regeneration in the number of red cells, blood haemoglobin concentration, mean cell volume and mean cell haemoglobin concentration and plasma iron is greatest in groups consuming a meat-based diet (containing 25% beef). Comparative vegetable- based diets that contained either non-haem iron or non-haem iron that had been fortified with the organic acid vitamin C had lower rates of regeneration.

The differences in the rates of regeneration are indicative of the differences in the bioavailability of the two forms of dietary iron. Meat iron is more bioavailable than non-meat iron found in vegetable material. This trend is maintained even when the bioavailability of non-meat iron is enhanced by the strategic use of vitamin C.

This suggests that the most effective way of reducing the incidence of iron deficiency is to include meat as part of diet. Unfortunately a large proportion of the world s population consume a vegetable based diet. Therefore this group should be encouraged to increase the consumption of foods containing vitamin C thereby enhancing the bioavailability of the non-meat iron. Although its use appears to be limited, increasing red blood cell production rather than iron retention.

Having established that the haem iron found mainly in meat regenerates the blood parameters more effectively; a second experiment then evaluated the effects of processing heat as most meat is cooked prior to consumption. Again the bioavailability of iron was assessed using a repletion study.

The findings suggest that iron deficient subjects consuming cooked meat diet have regeneration rates that are higher than those consuming either a diet containing raw uncooked meat. Which in turn has regeneration rates that are higher than a diet containing milk proteins. This suggests

that the cooking process is in some way beneficial, possibly by increasing the nutrient availability and digestibility of the diet.

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Appendices

Appendix 1

The production of red blood cells

[…] the production of red blood cells (erythropoiesis) takes place in the bone marrow. Red cell go through several stages of development before being released into the circulatory system.

- **Proerythroblast**

  ↓

- **Basophilic erythroblast**

  ↓

- **Polychromatophilic erythroblast**

  Haemoglobin synthesis begins.

  ↓

- **Acidophilic erythroblast**

  Haemoglobin synthesis is at maximum. The Acidophilic erythroblast ejects its nucleus and becomes a reticulocyte.

  ↓

- **Reticulocyte**

  This cell has the characteristic biconcave shape of the red blood cell. It contains about 34% haemoglobin and retains some mitochondria, ribosomes and endoplasmic reticulum. They pass from the red bone marrow into the blood stream by squeezing between the endothelial cells of blood capillaries. Developing into erythrocytes or mature red blood cells 1-2 days after their release from the red bone marrow.

  ↓

- **Erythrocyte** (mature red blood cell). […] Totara and Reynolds- Grabowski 1996.

Appendix 2

Erythropoiesis regulation. (Adapted from Totara and Reynolds- Grabowski 1996)

Normally erythropoiesis and red blood cell destruction proceed at the same pace. If the oxygen carrying capacity of the blood falls and oxygen delivery to the tissue is reduced then a negative feed back system increases red blood cell production.

- Homeostasis is disrupted.

  ↓
- Oxygen deliver to tissues is reduced.

  ↓
- Receptor cells located in the kidneys detect low oxygen level.

  ↓
- Cells secrete erythropoietin (EPO) a hormone that circulates to the red bone marrow.

  ↓
- Erythropoietin is detected in the red bone marrow.

  ↓
- Erythropoietin induces proerythroblasts in the red bone marrow to mature more quickly into reticulytes.

  ↓
- Increased numbers of reticulytes enter the circulatory system.

  ↓
- Thereby increasing the numbers of red blood cells in circulation.

  ↓
- Oxygen delivery to the tissues is increased.

  ↓
- Return to homeostasis.

Appendix 3

The red blood cell life cycle

Red blood cells have a limited life span of approximately 120 days as they lack a nucleus and other organelles that are needed to reproduce and carry out extensive metabolic activities. Worn out red cells are removed from the circulation and destroyed by fixed phagocytic macrophages in the spleen and liver and the breakdown products are recycled.

Figure 26 shows the formation and destruction of red blood cells and the recycling and the breakdown products.

1. Macrophages in the spleen, liver or red bone marrow phagocytize worn out red blood cells.

2. The globin and haem portions of the haemoglobin are split apart.

3. Globin is broken down into amino acids.

4. The amino acids are reused in the synthesis of other proteins.

5. The iron is removed from the haem portion.

6. The iron forms an association with a plasma protein called transferrin, which transports iron in the blood.

7. In muscle fibres, liver cells and macrophages of the spleen and liver, iron detach from transferrin and attaches to the iron storage proteins ferritin and haemosiderin.

8. Upon release from the storage site or absorption from the gastrointestinal tract iron attaches to transferrin.

9. It is then transported to the bone marrow, where red blood cell precursors take it up through receptor-mediated endocytosis.

10. Where it is then used in the production of new haemoglobin molecules.

11. Erythropoiesis in the red bone marrow results in the production of red blood cells, which enter the circulation system.

12. The non-iron portion of the haem is converted to biliverdin a green pigment.

13. Biliverdin is then converted into bilirubin, an orange pigment.

14. Bilirubin enters the blood and is transported to the liver.

15. Within the liver bilirubin is secreted by liver cells into bile, which is passed into the small intestine.

16. In the large intestine bacteria convert bilirubin into urobilinogen.

17. Some urobilinogen is absorbed back into the blood, converted into urobilin, a yellow pigment and excreted in urine.

18. Most urobilinogen is eliminated in faeces in the form of a brown pigment called stercobilin.

Figure 26: Formation and destruction of red blood cells and recycling of the haemoglobin components (From The principles of anatomy and physiology 8th edition. Totara and Reynolds-Grabowski. Pg 560.