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LIST OF ACRONYMS AND ABBREVIATIONS

AI	Adequate Intake
ANOVA	Analysis of Variance
AOAC	Association of Analytical Chemists
ARC	Agricultural Research Council
AUC	Area Under the Curve
BHT	Butylated Hydroxytoluene
CE	Capillary Electrophoresis
CEN	European Committee for Standardisation
CV	Coefficient of Variation
DALY	Disability Life Year
DM	Dry Matter
DRI	Dietary Reference Intake
EAR	Estimated Average Requirement
FAO	Food and Agricultural Organisation
FCR	Feed Conversion Ratio
FCS	Food Control System
FFP	Food Fortification Program
GC	Gas Chromatograph
HPLC	High Performance Liquid Chromatograph
ICN	International Congress on Nutrition
INP	Integrated Nutrition Program
IOM	Institute of Medicine
ISO	International Standards Organisation
IU	International Units

IVACG	International Vitamin A Consultative Group
LC	Liquid Chromatography
LLE	Liquid Liquid Extraction
LOD	Limit of Detection
MDGs	Millennium Development Goals
LOQ	Limit of Quantification
NFCS	National Food Consumption Survey
NFCS-FB	National Food Consumption Survey Fortification Baseline Study
NHLS	National Health Laboratory Services
PCA	Principal Component Analysis
PDA	Photo Diode Array
RAE	Retinol Activity Equivalent
RDA	Recommended Dietary Allowance
RE	Retinol Activity
RP	Reverse Phase
RSI	Recommended Safe Intake
SANAS	South African National Accreditation Services
SAVCG	South African Vitamin A Consultative Group
SD	Standard Deviation
SPE	Solid Phase Extraction
TRM	Treatment
UNICEF	United Nations Children Fund
UL	Upper Limit
VAD	Vitamin A Deficiency
WHO	World Health Organisation

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CHAPTER 1

THE STUDY IN PERSPECTIVE

"A knowledge of the chemical composition of foods is the first essential dietary treatment of disease or in any quantitative study of human nutrition"

(McCance and Widdowson, 1940)

1.1 Background to the Study

Vitamin and mineral deficiencies adversely affect a third of the world's people (Darnton-Hill, et al., 2005). The International Congress on Nutrition (ICN) World Declaration and Plan of Action for Nutrition recommended steps in order to eliminate iodine and vitamin A deficiencies before the end of this decade (FAO, 1992; Clarke, 1995). These pledges have been reaffirmed at the World Summit in Johannesburg, South Africa in September 2002 (United Nations, 2002). Food-based approaches were recognised by the ICN as the most effective way to address existing micronutrient deficiencies. These approaches can include strategies to assure dietary diversification, improved food availability, food preservation, nutrition education and food fortification (Clarke, 1995). A combination of food based strategies, food fortification and supplementation is advised (WHO, 2009).

Since 1994, remarkable progress has been made in reaching optimal levels of iodine nutrition in a majority of populations. Close to 70% of households in the developing world have access to iodised salt through iodine fortification. The World Health Organisation (WHO) database on iodine deficiencies shows that the number of countries with iodine deficiency as a public health problem has decreased from 110 in 1993 to 54 in 2003 (Mangasaryan et al., 2005; WHO, 2009). On the other hand little

progress has been made in eliminating vitamin A deficiency (VAD). According to WHO (2003) between 100 and 140 million children are vitamin A deficient. An estimated 250 000 to 500 000 vitamin A deficient children become blind every year, half of them dying within 12 months of losing their sight (WHO, 2009). To successfully combat VAD, short-term interventions and proper feeding in infancy must be supported by long-term sustainable solutions. These interventions include a combination of breastfeeding and vitamin A supplementation, coupled with enduring solutions, such as the promotion of vitamin A-rich diets and food fortification.

Fortification is defined by the Codex Alimentarius (1991) as the addition of one or more essential nutrients to a food, whether or not it is normally contained in the food, for the purpose of preventing or correcting a demonstrated deficiency of one or more nutrients in the population or specific population groups. Most staple foods are not complete foods and are deficient in one or more micronutrients. Food fortification of staple foods with micronutrients may help in overcoming deficiency problems in a population. Fortification has the advantage of requiring fewer changes in consumer behaviour and food habits than other interventions (Darnton-Hill and Nalubola, 2002). The major challenges involved in fortifying foods include the identification of suitable vehicles, selection of appropriate fortificant compounds, the level of fortification, determination of technologies to be used in the fortification process and the implementation of appropriate monitoring mechanisms to determine whether the goals of the programme are being met. Reliable methods for determining micronutrient status are required both in establishing the need for food fortification and in monitoring its nutritional impact (WHO, 2006).

A holistic approach to food fortification should be taken, and this emphasises many supporting activities that would facilitate efficacious fortification. Considerations other than only the methodologies employed in the addition of micronutrients to foods greatly

influence the potential of food fortification to meet its nutritional objectives. These include: current technologies for determining micronutrient status of target population groups; bioavailability of certain micronutrients in fortified foods and impact of traditional cooking and preparation practices on the stability of nutrients in fortified foods (Clarke, 1995).

1.2 The South African context

In South Africa, refined white maize meal is currently the main staple food due to consumer demand (NFCS, 2000). Ironically, the preferences of today's African consumers for white as opposed to yellow maize as is consumed by the rest of the world, was initially created by the influence of the British starch market. Since 1911, the British starch market provided a premium for white maize and local legislation was passed in some parts of Eastern and Southern Africa requiring that only white maize be accepted for export. The influence of mines, plantations, and cattle enterprises on to the local economy expanded the demand for food in the country. Eventually the domestic demand for maize grew as Africans left their farms to work on settler farms, in mines or industrial plants. Food consumption preferences were influenced by the rations that employers used as in-kind payments. Diets adapted as "people got used to what they consumed" (Shopo 1985 cited Smale & Jayne, 2003: 11).

Micronutrient deficiencies are prevalent in the country and primarily affect vulnerable groups such as children and women. The National Food Consumption Survey (NFCS) of 1999 showed that most children appear to consume a diet low in energy and poor in protein quality and micronutrient density. It also found that one out of two children aged 1-9 years have an intake of approximately less than half the recommended level for vitamin A, vitamin C, riboflavin, niacin, vitamin B_6, folate, calcium, iron and zinc. Iron

deficiency and anaemia are common problems among children in rural communities. Although anaemia could be a result of malaria and parasite infestations, dietary deficiency in iron is also a major concern (NFSC, 2000).

The NFCS findings support the results from the 1994 South African Vitamin A Consultative Group (SAVACG) survey among children 6-71 months which found that 33.3% of children are vitamin A deficient, a prevalence which indicates that vitamin A deficiency is a serious health problem in this country. The SAVACG survey also found a 21.4% prevalence of anaemia, 10% prevalence of iron deficiency and 5% prevalence of iron deficiency anaemia (SAVCG, 1996).

As part of a food based approach to alleviate micronutrient malnutrition the Directorate of Nutrition initiated a food fortification program (FFP). A multi-sectoral Food Fortification Task team (including members from UNICEF, Micro Nutrient Initiative, the National Chamber of Milling, the South African Chamber of Baking and independent millers) were tasked to investigate the critical components of such a food fortification program (FFP). The Council for Scientific and Industrial Research (CSIR) were contracted to conduct stability tests and sensory evaluation of fortified food vehicles for the FFP (Kuyper, 2000). Some of the concerns raised in the study were the level of fortification needed to compensate for losses due to storage, packaging and cooking. These concerns motivated the funding for the present project through the National Research Foundation.

1.3 Motivation for the study

Fortification of maize meal and white and brown bread flour with vitamin A, thiamine, riboflavin, niacin, pyridoxine, folic acid, iron and zinc became mandatory in South Africa

since 7 October 2003 (Department of Health, 2003). While it is generally possible to add a mixture of vitamins and minerals to relatively inert and dry foods such as cereals, interactions can occur between fortificant nutrients that adversely affect the sensory qualities of the foods (Allen et al., 2004; Clarke, 1995), or the stability of the nutrients (Mehansho et al., 2003; Allen et al., 2004; Clarke, 1995). There is a lack of knowledge regarding the quantitative impact of the interactions among nutrients added as a mixture, on the stability and absorption of the individual nutrients (Allen et al., 2004). The effects of cooking and exposing the fortification mix to moisture and heat for a period of time, also need to be ascertained. It is also important to consider the vehicle for fortification, in this instance, maize meal's inherent nutrients and anti-nutrients such as fibre and phytate and its interaction with the fortification mix.

Few vitamin A fortification programs have been appropriately evaluated, often because of resource constraints. Most of the evaluations that have been done relied on serum retinol concentrations to assess change in vitamin A status in individuals in response to an intervention. Because serum retinol concentration is not an optimal indicator for assessing change in status, the results of these evaluations are difficult to interpret (Vitamin A Tracer Task Force, 2004). Measuring the true change in vitamin A status in response to an intervention is important so that program managers and policy makers can avoid drawing incorrect conclusions about the efficacy or effectiveness of interventions. It is also important to understand if levels and vehicles identified are adequate to improve micronutrient status, and policies should be adopted to in order to ensure the effectiveness of fortification.

1.4 Objective of the study

The aim of the research described in this thesis was to quantify the content and relative absorption of vitamin A in fortified maize meal as purchased from the shelves of retail outlets, as well as in the cooked products that are traditionally prepared and consumed. These data will enable nutritionists and policy makers to make informed decisions on choice of fortificant as well as the vehicle and level of fortification.

The ultimate project objective is to provide policy makers with information that can assist them in implementing food policies leading to improved household food security and growth and well-being of South Africans. Access to such information will assist decision making towards improving efficiency in the application of the South African food fortification program.

1.5 Presentation and structure of the thesis

The structure and outline of the thesis is as follows:

CHAPTER 1: THE STUDY IN PERSPECTIVE

An overview of the study was provided in this chapter.

CHAPTER 2: LITERATURE REVIEW

A literature review is presented on vitamin A deficiency and fortification. This is followed by a discussion of factors that may have an influence on the success of such a program.

CHAPTER 3: VITAMIN A CONTENT OF FORTIFIED WHITE MAIZE MEAL AS PURCHASED AND CONSUMED IN SOUTH AFRICA

A method to determine vitamin A in maize meal was optimised and validated. The method was accredited by the South African National Accreditation Services (SANAS). This method was subsequently used to determine the vitamin A content of maize meal samples, as well as of the corresponding maize porridge samples. Retention of vitamin A in cooked porridge was calculated.

CHAPTER 4: EFFECT OF DIFFERENT MAIZE MEAL DIETS ON THE GROWTH AND VITAMIN A STATUS OF CHICKENS

The relative efficacy of the daily consumption of fortified maize meal in sustaining or improving vitamin A status was evaluated. Although children could be used to evaluate their vitamin A status after consumption of fortified maize meal, this was beyond the financial means of the project and such an approach also has limitations. Consequently, chickens were used as the biological model. Growth and vitamin A status were evaluated using the weight, feed conversion and liver retinol stores of the chickens on different diets over a six week period.

CHAPTER 5: DISCUSSION, CONCLUSION AND RECOMMENDATIONS

The last chapter summarises the main findings of the described research. The implications of these findings and recommendations to consider in the future are presented and discussed.

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CHAPTER 2

LITERATURE REVIEW

In this chapter a literature review is presented on vitamin A deficiency, fortification and factors that might have an influence on the success of such a program.

2.1 Introduction

The estimates from the World Health Organisation in the period 1995 - 2005 indicate that 190 million preschool children (~32%) and nearly 20 million pregnant women (~10%) are vitamin A deficient in low income countries (WHO, 2009). An estimated 250 000 to 500 000 vitamin A deficient children become blind every year, half of them dying within 12 months of losing their sight (WHO, 2003).

To successfully combat vitamin A deficiency (VAD), short-term interventions and proper feeding in infancy must be supported by long-term sustainable solutions. The solutions to nutritional well-being include a combination of breastfeeding and vitamin A supplementation, coupled with long-term food-based solutions, such as the promotion of vitamin A-rich diets and food fortification.

Breast milk is the main sources of vitamin A for infants. Poor maternal vitamin A status, and the resultant low breast milk retinol content are risk factors for the early onset of VAD in infants, as is early cessation of breastfeeding (Allen and Gillespie, 2001).

The most widely practised approach to control VAD in high-risk countries is the periodic delivery of Vitamin A supplements. While periodic vitamin A delivery in the community has been shown to reduce the risks of xerophthalmia or night blindness (by ~90%) and mortality (by ~23–30%) in young children, the reasons for the modest and transient effect in raising population serum retinol concentrations remain unclear. Many high-risk countries have also adopted the WHO policy of supplementing mothers with a 200 000 IU oral dose of vitamin A within six weeks after delivery to enrich the vitamin A content of their breast milk, although in practice coverage remains quite low (WHO, 2009).

Fortifying a widely consumed centrally processed food or condiment capitalizes on the production and distribution system of the food market to deliver low doses of vitamin A daily to a large number of people. Food fortification has many advantages: it is generally socially acceptable, it requires minimal changes in food habits, fortified foods usually costs <2% more than the cost of the unfortified food, its delivery system is already in place and it can become sustainable (Dary and Mora, 2002). The success of a fortification program depends among other factors on the mix of micronutrients and the concentration thereof in the fortified products. A number of aspects, including nutrient interactions, the stability of the specific micronutrients added to the food under anticipated conditions of storage and processing can all have an influence on the fortificant concentration.

In this chapter vitamin A deficiency and factors contributing thereto; vitamin A metabolism and absorption; factors influencing the vitamin A concentration in fortified products and measurements of relative bioavailability will be discussed.

2.2 Vitamin A and the isomers

Vitamin A is a generic term used for a group of structurally related chemical compounds known as retinoids. Retinoids refer to both naturally occurring and synthetic compounds with, or without, the biological activity of vitamin A (O'Byrne and Blaner, 2005). Figure 2.1 shows the chemical structures of some retinoids. The term vitamin A is often used as a general term for all compounds that exhibit the biological activity of retinol.

In vivo, vitamin A is generally found as the free alcohol form (retinol) or esterified with a fatty acid (retinyl ester). All-*trans*-retinol is by definition vitamin A. The vitamin is available in pure form by chemical synthesis or as vitamin A palmitate or acetate. It is a pale yellow solid, which dissolves freely in oils and fats, but is insoluble in water (Fox and Cameron, 1995).

2.2.1 Sensitivity of Vitamin A

Vitamin A is affected by pH, enzymatic activity, light and oxidation associated with the double bond system (DSM/USAID, n.d.b). In Table 2.1 a summary of Vitamin A sensitivities compared to other vitamins is presented. Vitamin A is quite stable when heated to moderate temperatures in the absence of oxygen and light. Overall loss of activity during anaerobic heating may range from 5-50%, depending on time, temperature and nature of the retinoids. In the presence of oxygen and light, there can be extensive loss of vitamin A activity through oxidation. The presence of trace metals accelerates this reaction (Ottaway, P.B. cited Mehansho et al., 2003).

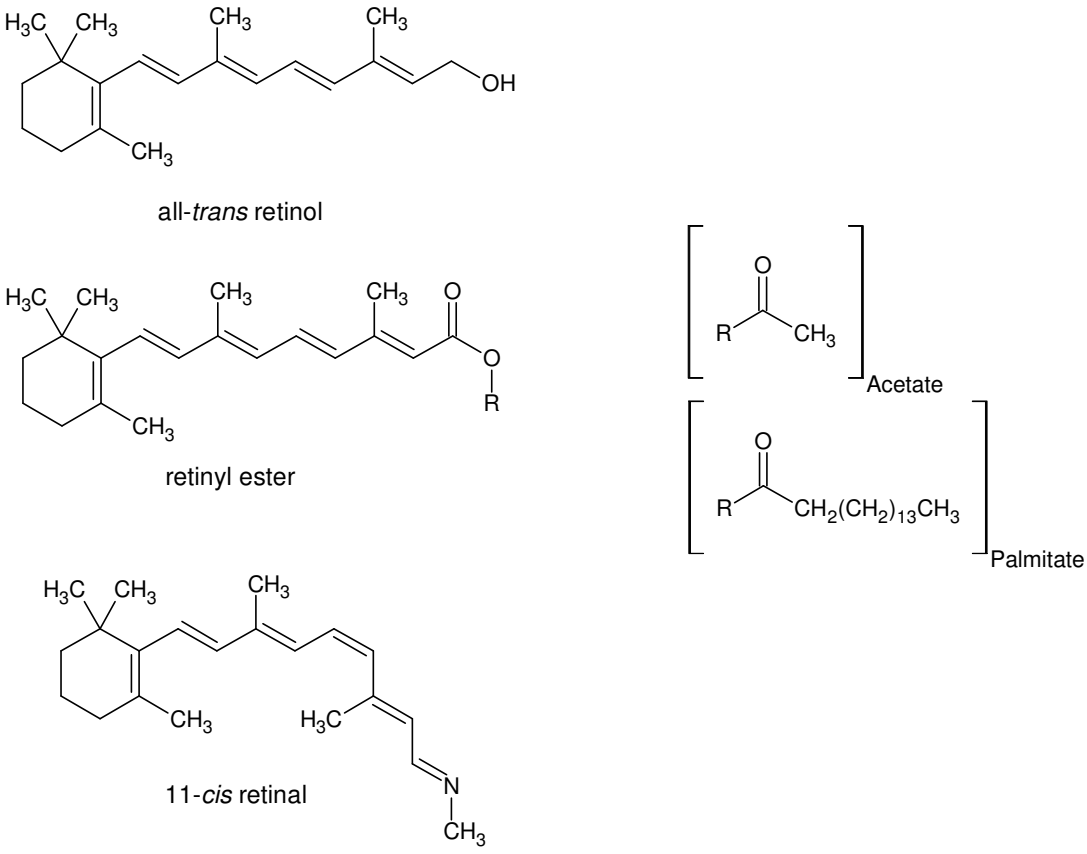

Figure 2.1: Chemical structures of different retinoids. All-*trans*-retinol is by definition vitamin A. When a fatty acyl group is esterified to the hydroxyl terminus of all-*trans*-retinol, a storage form of retinol, the retinyl ester is formed. The most abundant retinyl esters are those of palmitic, oleic, stearic and linoleic acids. Retinyl acetate and palmitate are often used as dietary supplements, but do not occur naturally. Retinol can be reversibly oxidized to retinal, which as the 11-*cis* isomer is essential for the visual cycle (O'Byrne and Blaner, 2005)

Table 2.1: Sensitivity of vitamin A compared to other vitamins (DSM/USAID, n.d.b)

	Light	Oxidizing agents	Reducing agents	Heat	Humidity	Acids	Alkalis
Vitamin A	+++	+++	+	++	+	++	+
Vitamin D	+++	+++	+	++	+	++	++
Vitamin E	++	++	+	++	+	+	++
Vitamin K	+++	++	+	+	+	+	+++
Vitamin C	+	+++	+	++	++	++	+++
Thiamine (Vit B_1)	++	+	+	+++	++	+	+++
Riboflavin (Vit B_2)	+++	+	++	+	+	+	+++
Niacin	+	+	++	+	+	+	+
Pyridoxine (Vit B_6)	++	+	+	+	+	++	++
Cyanocobalamin (Vit B_{12})	++	+	+++	+	++	+++	+++
Pantothenic Acid	+	+	+	++	++	+++	+++
Folic Acid	++	+++	+++	+	+	++	++
Biotin	+	+	+	+	+	++	++

+ Hardly or not sensitive ++ Sensitive +++ Highly sensitive

In dehydrated foods, vitamin A and provitamin A are highly susceptible to loss by oxidation. The extent of this loss depends on the severity of the drying process, protection provided by packaging materials and conditions of storage. Vitamin A in pure form is unstable in the presence of mineral acids but stable in the presence of alkali.

Naturally occurring vitamin A is insoluble in water but soluble in oil. In the natural form the vitamin has limited applicability in fortification. Vitamin A as fortificant are commercially available in a wide range of forms adapted for use under various conditions as presented in Table 2.2.

Table 2.2: Commercially available forms of vitamin A, their characteristics and their main applications (WHO, 2006).

Product	Characteristics	Application(s)
Oily vitamin A acetate	Retinol ester of acetic acid which may be stabilized with especially antioxidants	Fortification of fat-based foods, margarine and dairy products
Oily vitamin A palmitate	Retinol ester of palmitic acid which may be stabilised with antioxidants	Fortification of fat-based foods, especially margarine and dairy products
Oily vitamin A palmitate or acetate with vitamin D3	Retinol ester and cholecalciferol mix, stabilised with antioxidants	Fortification of fat-based foods, especially margarine and dairy products where the combination of both vitamins is required
Dry vitamin A palmitate or acetate	Vitamin A embedded in a water-soluble matrix (e.g. gelatin, gum acacia, starch) and stabilised with antioxidants	Fortification of dry food products, (i.e. flour and dry milk, beverage powders) and fortification of water-based foods
Dry vitamin A palmitate or acetate with vitamin D_3	Vitamin A and vitamin D_3 embedded in a water-soluble matrix (e.g. gelatin, gum acacia, starch) and stabilised with antioxidants	Fortification of dry food products, (i.e. flour and dry milk, beverage powders) and fortification of water-based foods

For use in fat or oil based foods such as margarines, oils and dairy products, vitamin A, in the acetate or palmitate form, has been used. These forms are stabilised with a mixture of phenolic antioxidants or with tocopherols. For mixing with dry products, a dry form of the fortificant is required with the appropriate size and density. Encapsulation of the vitamin in a more hydrophilic coat is commonly practised in order to achieve a more water dispersible product. Some materials used in encapsulation are gum acacia, starch and gelatin. These dry forms of the vitamin are also stabilised using tocopherols or phenolic antioxidants (Clarke, 1995; WHO, 2006). Vitamin A compounds needed for fortification of dry matrixes (e.g. flour and sugar) are at least four times more expensive than the oily forms, and their stability is inferior (Dary and Mora, 2002).

According to the South African fortification regulations a protected, stabilized vitamin A palmitate containing 75 000 µgRE activity per gram fortification mix must be used (Department of Health, 2003).

2.3 Vitamin A Metabolism and Deficiency

2.3.1 The role of Vitamin A in human metabolic processes

Although an essential nutrient needed in only small amounts, vitamin A is necessary for normal functioning of the visual system; growth and development; and maintenance of epithelial cellular integrity, immune function and reproduction. Vitamin deficiency disorders occur when body reserves are depleted to the limit at which physiological functions are impaired. Vitamin A in the diets of most human communities comes from a very wide variety of plant and animal sources (FAO, 2001). In the more industrialised countries over two-thirds of dietary vitamin A is derived from animal sources as preformed vitamin A, whereas in developing countries, communities depend primarily on provitamin A carotenoids from plant sources (Ahmed and Darnton-Hill, 2004). In an effort to satisfy energy needs, poor populations may have chosen diets of lesser quality and variety, which would increase the risk of multiple micronutrient deficiencies (West and Sucheta, 2010).

Provitamin A carotenoids is the collective term for all the carotenoids that can be converted to retinoids by humans and some animals (O'Byrne and Blaner, 2005). However, the conversion and bioavailability of the provitamin A carotenoids are much less efficient than retinol (Van Lieshout and West, 2004).

Preformed vitamin A in animal foods occurs as retinyl esters of fatty acids in association with membrane-bound cellular lipid and fat-containing storage cells. Normal digestive processes free vitamin A from embedding food matrices. Vitamin A is absorbed more efficient from animal products than from vegetable tissues. Retinyl esters are hydrolysed and the retinol is incorporated into lipid-containing, water-miscible micellar solutions. Products of fat digestion (e.g., fatty acids, monoglycerides, cholesterol, and phospholipids) and secretions in bile (e.g., bile salts and hydrolytic enzymes) are essential for the efficient solubilisation of retinol. Retinol is trapped intracellularly by re-esterification or binding to specific intracellular binding proteins (O'Byrne and Blaner, 2005). Retinyl esters together with other lipids are incorporated into chylomicrons, excreted into intestinal lymphatic channels and delivered to the blood through the thoracic duct. If not immediately needed, retinol is re-esterified and retained in the fat-storing cells of the liver (FAO, 2001).

Vitamin A functions at two levels in the body. The first is in the visual cycle in the retina of the eye; the second is in all body tissues systemically to maintain growth and the soundness of cells. The growth and differentiation of epithelial cells throughout the body are especially affected by vitamin A deficiency (VAD). The immune system is also compromised by direct interference with production of some types of protective secretions and cells (FAO, 2001).

2.3.2 Bioavailability of vitamin A

The amount of a nutrient absorbed from the gut which becomes available to tissues is referred to as bioavailability (Van Lieshout and West, 2004). Preformed vitamin A is absorbed in the small intestine. The bioavailability of retinol is generally high;

ranging from 70 – 90% (Dary and Mora, 2002; Otten, Hellwig and Meyers, 2006), while that of carotenoids is lower and is affected by various factors (Castenmiller et al., 1999; Van het Hof et al., 2000). Different carotenoids have different levels of vitamin A activity depending upon the efficiency of their absorption and the rate of their conversion to vitamin A. Whereas 1 retinol equivalent (RE) is equal to 1 mg of all-*trans* retinol, the same level of vitamin A activity requires 6 mg of beta-carotene and 12 mg of other carotenoids with vitamin A activity (West, Eilander and Van Lieshout, 2002). By the late 1990's, conversion factors for estimating vitamin A obtained from plant foods were revised from 6:1 to 12:1 (µg ß-carotene:retinol activity equivalent (RAE)) by the US Institute of Medicine (IOM). De Pee, West and colleagues proposed a conversion factor of 21:1 for a mixed diet (12:1 for fruits and 26:1 for vegetables (De Pee et al., 1998; IVACG, n.d.). The cost factor in using carotenoids as the source of vitamin A activity in fortification is generally considered prohibitive (Clarke, 1995).

When vitamin A intake is adequate, more than 90% of total body vitamin A is located in the liver, which releases the nutrient into the circulation. Factors such as dietary fat, intestinal infections, the food matrix, and food processing can affect the absorption of vitamin A by the body. Dietary fat appears to enhance absorption, whereas absorption is reduced in individuals with diarrhoea, intestinal infections and infestations (Blomhoff, 1994; Herrero-Barbudo, et al., 2006; Edem, 2009).

2.3.3 Dietary Requirements and Toxicity

2.3.3.1 Definitions of Recommended Dietary Allowance, Recommended Safe Intake and Daily Reference Intake

The mean requirement for an individual is defined by the FAO as the minimum daily intake of vitamin A as presented in Table 2.3 to prevent xerophthalmia in the absence of clinical or sub-clinical infection. This intake should account for proportionate bioavailability of preformed vitamin A (about 90%) and pro-vitamin A carotenoids from a diet that contains sufficient fat (e.g., at least 5–10g). The required level of intake is set to prevent clinical signs of deficiency, allow for normal growth, and reduce the risk of vitamin A–related severe morbidity and mortality on a population basis. It does not allow for frequent or prolonged periods of infections or other stresses (FAO, 2001). The safe level of intake for an individual is defined as the average continuing intake of vitamin A required to permit adequate growth and other vitamin A–dependent functions and to maintain an acceptable total body reserve of the vitamin. This reserve helps offset periods of low intake or increased need resulting from infections and other stresses. Estimates for the requirements and recommended safe intakes of all age groups are estimates derived from vitamin A requirements/body weight/day for late infancy (FAO, 2001).

Recommended dietary allowances (RDA) as defined by the FAO and WHO are set to meet the needs of almost all (97-98%) individuals in a group. For healthy breastfed infants, the adequate intake (AI) is the mean intake. The AI for other life stage and gender groups is believed to cover the needs of all individuals in the group, but a lack of data prevents being able to specify with confidence the percentage of individuals covered by this intake (FAO, 2001).

Table 2.3: FAO estimated mean requirement and safe level of intake for vitamin A (FAO, 2001)

Age Group	Mean Requirement (µgRE/day)	Recommended Safe Intake (µgRE/day)
Infants		
0-6 months	180	375
7-12 Months	190	400
Children		
1-3 years	200	400
4-6 years	200	450
7-9 years	250	500
Adolescents		
10-18 years	330 – 400	600
Adults		
Females: 19-65 years	270	500
Males: 19-65 years	300	600
Elderly		
65+ years	300	600
Pregnant Women	370	800
Lactating Women	450	850

The Food and Nutrition Board of the National Academies' Institute of Medicine (IOM), with support from the US and Canadian Governments developed a new, broader set of dietary reference values known as the Dietary Reference Intakes (DRIs). See Table 2.4. The DRIs expand upon and replace the RDAs with four categories of values intended to help individuals optimise their health, prevent disease and avoid consuming too much of a nutrient. The reference values include the estimated average requirement (EAR), the recommended dietary allowance (RDA), the adequate intake (AI) and the tolerable upper intake level or upper limit (UL). The following definitions and criteria are used:

- The estimated average requirement (EAR) is the average daily nutrient intake level that is estimated to meet the nutrient needs of half of the healthy individuals in a life stage or gender group. Metabolic weight ($kg^{0.75}$) ratio method was used to extrapolate the data from adults (IOM, 2001).
- The definition of the RDA is the same as described above.

- The adequate intake (AI) is a recommended average daily nutrient intake level based on observed or experimentally determined approximates of nutrient intake by a group of apparently healthy people who are assumed to be maintaining an adequate nutritional state (IOM, 2001).
- The upper limit (UL) is the maximum level of daily nutrient intake that is likely to pose no risk of adverse effects. Unless otherwise specified, the UL represents total intake from food, water, and supplements. The UL for vitamin A applies only to preformed vitamin A. It does not apply to vitamin A derived from carotenoids (IOM, 2001).

Table 2.4: Dietary Reference intakes (DRIs) for Vitamin A by life stage group (IOM, 2001)

Age Group	EAR (µg/day)	RDA (µg/day)	AI (µg/day)	UL (µg/day)
Infants				
0-6 months			400*	600
7-12 Months			500*	600
Children				
1-3 years	210	300		600
4-8 years	275	400		900
9-13 years	445	600		1 700
Males				
14-18 years	630	900		2 800
19-50 year	625	900		3 000
>50 years	625	900		3 000
Females				
14-18 years	485	700		2 800
19-50 year	500	700		3 000
>50 years	500	700		3 000
Pregnant				
Women	530	750		2 800
≤18 years	550	770		3 000
19-50 years				
Lactating				
Women	885	1 200		2 800
≤18 years	900	1 300		3 000
19-50 years				

*For healthy breastfed infants, the AI is the mean intake.

As seen in Table 2.3 and Table 2.4, lactating women require the highest vitamin A intake. The mean requirement estimated by the FAO (Table 2.3) is generally lower than the EAR (Table 2.4) as determined by the IOM; with the most significant difference for pregnant and lactating women. The IOM also determines RDA values that are generally higher than the Recommended Safe Intakes (RSI) estimated by the FAO. The main reason for the higher EAR and DRI values from the IOM is the fact that the estimates was made on metabolic weight and not on total body weight as was used by the FAO.

2.3.3.2 Toxicity

Because vitamin A is fat soluble and can be stored, primarily in the liver, routine consumption of large amounts of vitamin A over a period of time can result in toxic symptoms. A review of the latest available information by a WHO Expert Group recommended that daily intakes in excess of 3 000 µg (10 000 IU) or weekly intakes in excess of 7 500 µg (25 000 IU), should not be taken (FAO, 2001). Vitamin A fortification of foods in dosages not exceeding the RDA does not cause toxic effects (Lotfi et al., 1996).

2.4 Vitamin A Deficiency

Vitamin A deficiency (VAD) is, after protein-energy malnutrition and iron deficiency anaemia, the nutritional health problem of highest public health significance in developing countries. Globally, more than 200 million children are vitamin A deficient, and VAD is still the leading cause of blindness in children. Women in developing

countries are also at risk of VAD, especially during pregnancy and lactation (Ahmed and Darnton-Hill, 2004).

In South Africa, 1 in 3 preschool children has a marginal vitamin A status (serum vitamin A concentration <0.7 µmol/L) (SAVACG, 1996). Normal serum vitamin A concentration for pre-school children is between 0.63 – 1.75 µmol/L (WHO, 1996.). 55–68% of children aged 1–9 years consume <50% of the recommended dietary intake of vitamin A (700 µg retinol equivalents) (NFCS, 2000). Figure 2.2 shows the prevalence of VAD in the nine different provinces in South Africa. Children living in rural areas are the most affected (SAVACG, 1996; NFCS, 2000). VAD is caused by a habitual diet that provides too little bioavailable vitamin A to meet physiological needs.

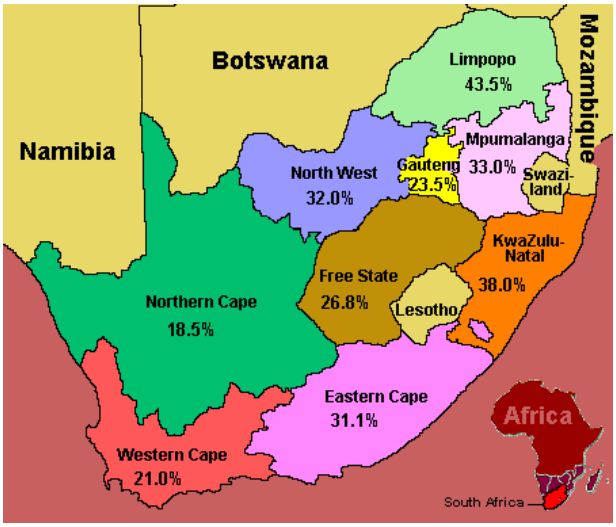

Figure 2.2: Prevalence of Vitamin A Deficiency in 6-71 month old children in South-Africa in 1994 as determined by the SAVACG study (SAVACG; 1996)

VAD is associated with a higher risk of death in preschool-age children, presumably because of vitamin A's role in the immune function and in maintaining the integrity of epithelial tissue. Supplementation with vitamin A reduces the risk of child mortality and may reduce maternal mortality. It also reduces the risk of severe diarrhoea and

measles, both of which are important and sometimes serious illnesses in developing countries. Because the vitamin A content of breast milk is often low in vitamin A-depleted women, infants of these women are at greater risk of becoming VAD early in life. If left untreated, this can result in a vicious cycle of deficiency that is not resolved (Van Lieshout and West, 2005).

Malnutrition causes the loss of about 140 million disability adjusted life years. The Disability Life Year (DALY) is the only quantitative indicator of burden of disease that reflects the total amount of healthy life lost, to all causes, whether from premature birth mortality or from some degree of disability over a period of time. DALY's are a quantitative way to compare the effect of various diseases on societies. Figure 2.3 shows that almost 25 million DALY's are lost due to VAD worldwide. In Africa alone, VAD causes the loss of almost 17 million DALY's (WHO, 2002); and in South Africa between 86 388 and 136 009 DALY's are lost (Nojilana et al., 2007).

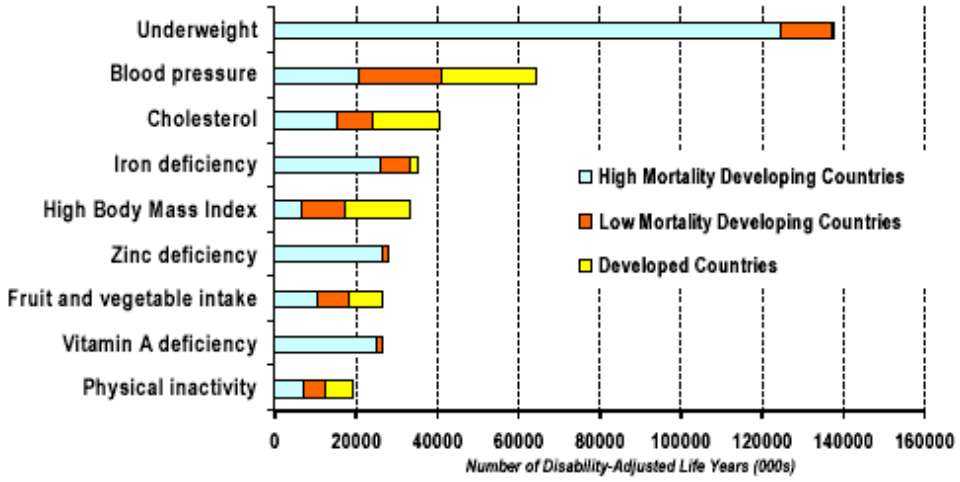

Figure 2.3: Disease burden (DALY's) in 2000 attributable to undernutrition and diet-related risks and physical inactivity (WHO, 2002)

It is understood that food consist of many nutrients, and that when communities are at risk for vitamin A deficiency, they may be at risk of other nutrient deficiencies as

well. Correcting VAD in populations at risk of deficiency is an investment to improve human development.

2.4.1 Strategies for controlling Vitamin A deficiencies

Increasing the efficacious nutrient supply, by using food-based approaches or by using pharmanutrient approaches, can control deficiency of vitamin A, as well as deficiency of other micronutrients. Another strategy for controlling VAD is to reduce the nutrient requirements, by for example, controlling infection (Van Lieshout and West, 2004).

Food-based interventions are viewed as those most likely to be sustained, provided the culture and ecology of the vitamin A-containing foods is addressed in programs based in agriculture, food processing, social marketing and public health education (Blum et al., 1997). When considering food approaches for combating vitamin A deficiency, it is necessary to take into account whether the efficacious nutrient supply can be met. Figure 2.4 illustrates the balance between the supply of nutrients and the requirements thereof. The efficacious nutrient supply depends on:

- Amount of foods containing vitamin A or provitamin A carotenoids consumed
- Vitamin A or provitamin A carotenoid content of each food consumed, and
- Bioefficacy of vitamin A or provitamin A carotenoids in the food consumed (Van Lieshout and West, 2004).

By far the most efficient food based approach for increasing the nutritional status of the nation is through fortification of widely consumed and accessible staple foods (Randall, 2001).

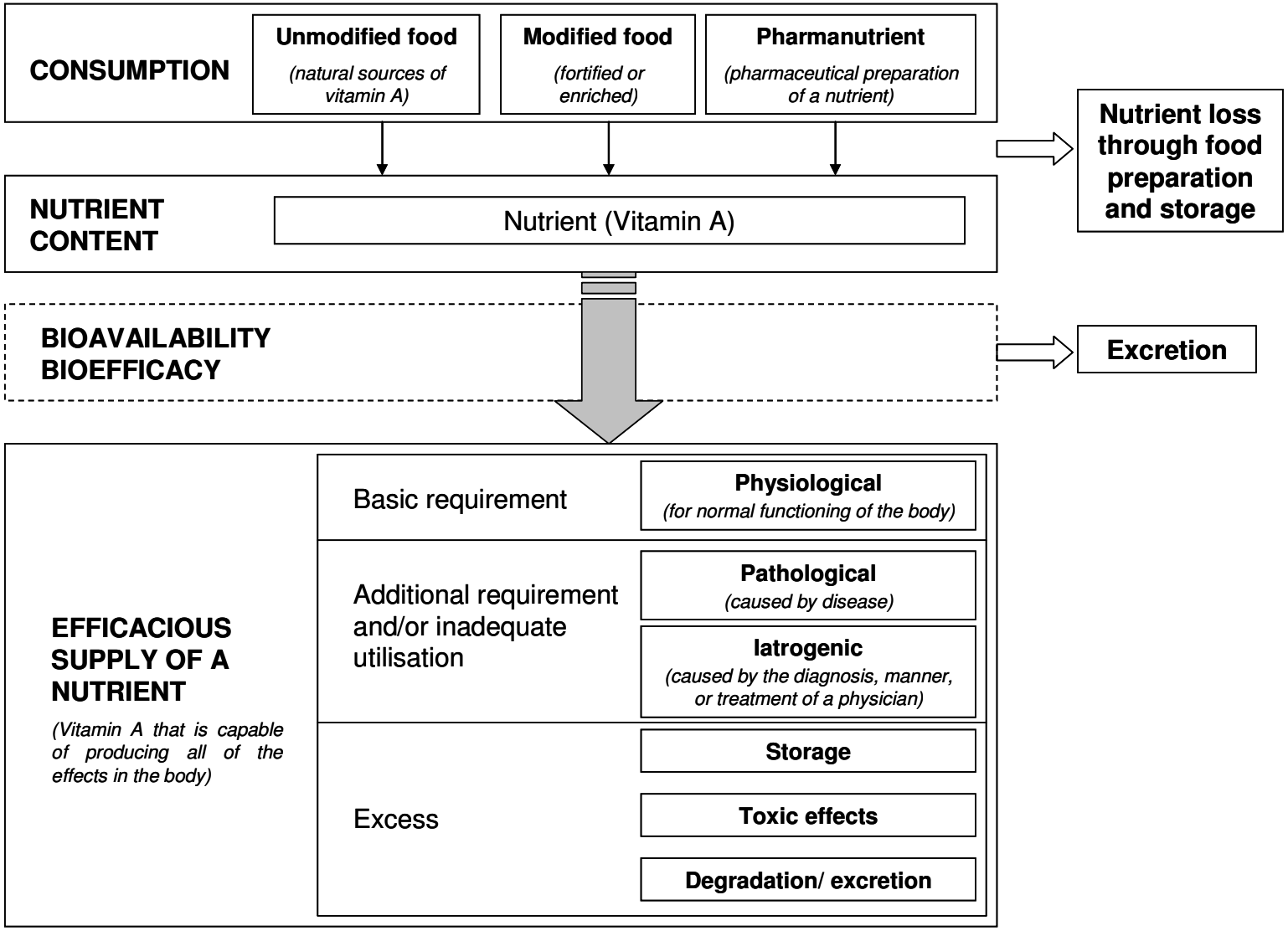

Figure 2.4: Balance between the supply of nutrients and requirements (Van Lieshout and West, 2005)

2.5 Fortification of staple foods with Vitamin A

Food fortification refers to the addition of micronutrients to foods during the production process. If fortified foods are consumed on a regular basis they will maintain body stores of nutrients more efficiently and more effectively than intermittent supplements. Fortification generally aims to supply micronutrients in amounts that approximate to those provided by a good, well-balanced diet. Consequently, fortified staple foods will contain “natural” or near natural levels of micronutrients, which may not necessarily be the case with supplements (WHO, 2006). Fortification of widely distributed and widely consumed foods has the potential for improving the nutritional status of a large proportion of the population. Fortification of food with vitamin A and its distribution are most feasible where the processed food industry is well-developed and supported. That may not be the case in resource-poor areas where vitamin A is lacking in the diet, deficiency is most extreme and various barriers exist for the most vulnerable to access fortified food (Trowbridge et al., 1993). An example in South Africa is food produced on farms which probably is used as in lieu of monetary payment that escapes mandatory fortification. This contributes to the finding that children on commercial farms are the worst fed as was found by the NFSC (NFSC, 2000).

To have a sustained impact on VAD, policy makers and program planners in agriculture and health must understand the nature of the fortificant (vitamin A), the food that is to be fortified (maize meal), methods of preparation and conservation of the food. Such knowledge will improve the dietary quality and quantity of vitamin A in fortification programs.

2.5.1 Maize meal as a vehicle for micronutrient fortification

The NFCS recommends maize meal (super, special and sifted) as one of the suitable vehicles for mandatory multiple micronutrient fortification (NFCS, 2000):

- Maize meal offers the best potential to deliver micronutrients to the widest spectrum of South Africans;
- Consumption among children is high, especially among 1-3 year-olds;
- 96% of maize meal is purchased from retailers;
- Production is relatively centralised with seven major companies dominating South Africa's maize milling industry, and contributing to about 90% of the domestic maize meal market. The three kinds of maize meal produced (from the most highly to the least processed) are: super, with a low extraction rate and high price; special, with an intermediate extraction rate and an intermediate price; and sifted maize with a very high extraction rate and low price (Bekker, 2004).

During the industrialised milling process many of the micronutrients concentrated in the outer layers of the maize kernel are removed (DSM/USAID, n.d.a), resulting in a highly refined product that is practically nothing more than pure starch. Refer to addendum A for the nutrient content of unfortified white maize meal. Refined white maize meal is the main staple food due to consumer demand (Shopo 1985 cited Smale and Jayne, 2003).

2.5.2 Vitamin A as a fortificant

Several forms of vitamin A are available for food fortification. These include retinyl acetate, retinyl palmitate, and provitamin A (ß-carotene). ß-carotene has an intense

orange colour that makes it unsuitable as a fortificant for many foods, but can be used to give an orange-yellow colour to margarines and beverages. The retinyl esters are available in an oil-soluble form (for fortification of oils and fats), spray-dried (for flours and powdered milk) and as water-dispersible beadlets (for fortification of sugar and other water soluble foods). A special coated, protected form of retinyl palmitate, often generically referred to as SD250, is the recommended form of vitamin A for flour fortification because it is considered to be the most stable in this application. This product contains encapsulates and antioxidants that differ between manufacturers, making it impractical to specify its exact composition. The stability of vitamin A in these commodities was found to be surprisingly good, with over 95% retained after nine months. There were additional losses during milling and baking, so that about 80% of the added vitamin A is actually consumed. Lower retentions, as low as 50%, can occur in non-bread baked products and maize meal (Johnson, Mannar, and Ranum, 2004).

2.5.3 Factors that affect nutrient delivery in fortification of maize meal

The success of a fortification program depends on a number of technical aspects, including nutrient interactions, the stability of micronutrients added to the food under anticipated conditions of storage and processing (food preparation at the household level) and bioavailability of the nutrients. Prior to selecting the fortificant(s), it is important to consider the factors affecting its/their stability (Figure 2.5). Physical and chemical factors include high temperatures, moisture, exposure to air or light, and acid or alkaline environments. The exposure of the fortificant to any of these factors during food processing, distribution, or storage affects its stability.

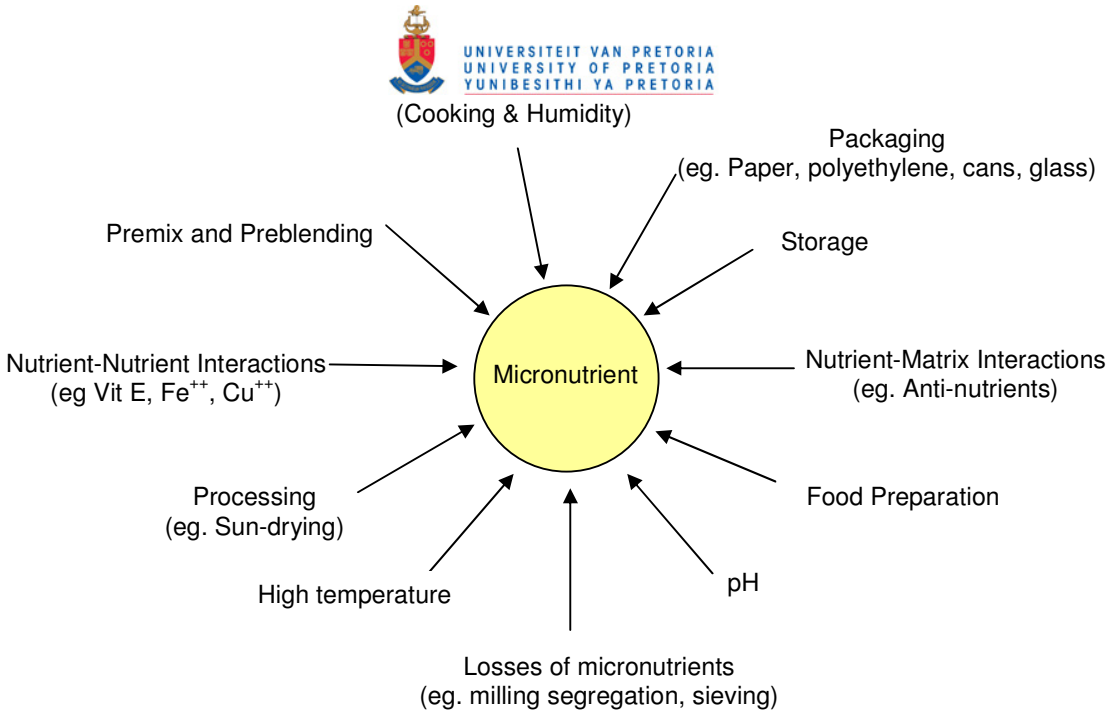

Figure 2.5: Physical and chemical factors influencing the stability of nutrients.

2.5.3.1 Nutrient-nutrient interactions

When more than one fortificant is being added to a particular vehicle, consideration must be given to the interactions, both positive and negative tat may occur. The presence of vitamin E has been shown to increase the bioavailability of vitamin A. One explanation for this is that tocopherol as a lipid phase antioxidant, stabilises vitamin A in the gastrointestinal tract (Clarke, 1995). On the other hand, the degradation (autoxidation) of vitamin A is accelerated by the presence of bioavailable iron as fortificant (Mehansho, et al., 2003; WHO, 2006), as well as other trace elements such as copper (Wirakartakusumah and Hariyadi, 1998). In South Africa “electrolytic iron" as elemental iron powder is included in the fortification premix as regulated by the Foodstuffs, Cosmetics and Disinfectants Act (Department of Health, 2003).

2.5.3.2 Nutrient-matrix interactions

Besides nutrient - nutrient interactions, other components of the food matrix may also affect the functionality of the fortificant. Selection of the vehicle in fortification programs must be such as to avoid reduced bioavailability of nutrients due to the presence of anti-nutritional compounds (Clarke, 1995). The naturally occurring anti-nutrients in maize meal such as fibres and phytates inhibit the absorption of trace metals (iron and zinc) (Welch, 1997). Dietary fibre reduces the bioavailability of vitamin A if consumed within the same meal (Lotfi et al., 1996). Vitamin A prevents the inhibitory effect of phytates and polyphenols on iron absorption (Garćia-Casal et el., 1998). The effect of phytates on vitamin A absorption and/or stability is not indicated in the literature and needs further investigation.

2.5.3.3 Moisture

Moisture contents in excess of about 7-8% in a food are known to adversely affect the stability of vitamin A. Beyond the critical moisture content, there is a rapid increase in water activity, which permits various deteriorative reactions to occur (Clarke, 1995). The moisture level of South African unfortified super maize meal, unfortified special maize meal and unfortified sifted maize meal is 12.0%, 11.6% and 11.9% respectively (Wolmarans, Danster and Chetty, 2005). A 6.5% moisture content of maize grits showed hardly any loss, whereas 11.4% moisture resulted in a loss of one-fifth of vitamin A (Cort et al., 1976, Lotfi et al., 1996).

2.5.3.4 Temperature

Vitamin A is stable under an inert atmosphere; however, it rapidly loses its activity when heated in the presence of oxygen, especially at higher temperatures (Lešková, 2006). Temperature may have an effect during storage as well as food preparation and is discussed under these headings (See 2.5.3.10 and 2.5.3.11).

2.5.3.5 pH

The stability of vitamin A is also affected by acidity. Below a pH of 5.0, vitamin A is unstable (Wirakartakusumah and Hariyadi, 1998). Increasing the acidity of food through fermentation is often used as preservation method. An example of a fermented/beverage that is popular in many parts of southern Africa, including South Africa, is *mahewu*, *amahewu*, also known as *magou* or *mageu.* Mageu is prepared by the fermentation of maize with lactic acid bacteria (Byaruhanga, Bester, and Watson, 1999). Lactic acid bacteria fermentation can cause a pH < 4.0 (Mensah, 1997).

2.5.3.6 Losses of added micronutrients

Some of the added micronutrients are lost during the milling process due to a combination of exposure to heat, oxygen and light. Some of the very light or small particle size materials with a large surface area may be physically removed with the dust during pneumatic suction, while larger particles may be removed by sieving. This can present as low values of vulnerable micronutrients (vitamin A, riboflavin) (Johnson, Mannar and Ranum, 2004). When mixing dry fortificants with dry foods, careful selection of the physical characteristics of the fortificant compound is

important to ensure adequate mixing and to minimise segregation on storage (Clarke, 1995).

2.5.3.7 Premix and preblend considerations

This is especially important in small-scale fortification. The concentrated premixes made for large scale fortification can be used in small scale fortification once they are properly diluted to a preblend. The cereal being fortified is used as the diluent. The dilution factor will be determined by the weight of cereals typically processed for each customer at the small mill. This can range from 2 to 20 kg at different mills. A fortification preblend has a much shorter shelf life than the parent premix – typically a few weeks rather than years – so it must be made in limited quantities in close proximity to the site where it will be used (Johnson, Mannar and Ranum, 2004).

2.5.3.8 Effect of further processing

In some cases the staple food that is brought to the small mill may need further processing before it is cooked at home. For example, de-hulled maize is brought to the mill for milling, but after the milling the maize meal is still moist. In this case the maize meal is spread out on mats in the sun to dry. Figure 2.6 shows this practise in the rural area near Giyani, Limpopo, South Africa. Sun-drying will effectively destroy most of the added vitamin A, riboflavin and folic acid (Johnson, Mannar and Ranum, 2004).

Figure 2.6: Sun-drying of hammer-milled maize meal in a rural village near Giyani

2.5.3.9 Packaging

Products that are improperly packaged and subsequently transported over long distances under hot and humid conditions experience micronutrient losses.

Packaging selection is greatly influenced by shelf-life considerations and cost. Vitamin A must be protected from oxygen and light. Amber glass containers are the best choice for these fortified products because they are not permeable to oxygen and protect against light. However, glass is heavy, fragile, and expensive, so plastics are often used instead. Oxygen readily passes through plastic and will come into contact with the product. Light-proof containers, for example, dark glass or dark plastic, cans, and aseptic boxes will minimize the exposure to light. Because of high costs and the lack of availability of packaging material in developing countries, packaging assumes great importance and should be a major factor that is taken into account at the beginning of a fortification program (Johnson, Mannar and Ranum, 2004). For example, loss of vitamin A in sealed cans of oil is minimal, while losses from fortified cereals or fortified sugar can be in the order of 40% depending on ambient conditions and storage time (Allen et al., 2004).

Guidelines of the Micronutrient Initiative (MI) for premix packaging indicate that it should be packaged in air and watertight containers well protected from exposure to light. Typical packaging is a polyethylene bag inside a heavy, cardboard box, fibre cartons or metal containers (Johnson and Philar, 2005). The package should be such that the bag can be easily resealed and the box closed after a portion of the product has been removed. Premixes should be kept in their original containers in a cool dry place prior to use. Once opened exposure to light and air should be minimised to prevent product degradation (Johnson, Mannar and Ranum, 2004).

In the CSIR final report on the stability tests and sensory evaluation of fortified food vehicles for the South African National Food Fortification Program it was found that during storage the stability of vitamin A in raw super maize meal was better in polyethylene bags than in paper bags (Kuyper, 2000). However, maize meal is mostly sold as large volumes in polyethylene bags, but in small volumes in paper bags. Figure 2.7 shows examples of various packagings.

Figure 2.7: Examples of maize meal packaging (25kg and 12,5kg in polyethylene bags and 1kg in a paper bag) as presented to consumers.

2.5.3.10 Effect of storage

The stability of micronutrients in fortified maize flour stored at room temperature is good. One study showed that yellow maize flour retained all its vitamin B_6, over 95% of vitamins A, B_1, and B_2, and about 85% of folic acid activity after six months storage at room temperature (Ranum, 1999). Flour enriched with a vitamin-mineral premix by Cort et al. (1976) also demonstrated excellent stability on storage at room temperature. Under conditions of accelerated storage at elevated temperature (45°C), however, there was substantial loss of vitamin A beyond 4 weeks of storage. Parrish et al. (1980) also reported good stability of enriched wheat flour stored at room temperature, but losses of about 50% in flour stored at 40°C for 6 months. Warm and humid storage conditions adversely affect the stability of some micronutrients, such as vitamin A (DSM/USAID, n.d.a). This must be considered in humid environments where warehouses are not climatically controlled and temperatures can rise to 45°C as often happens in certain rural parts of the country.

2.5.3.11 Food preparation

A second type of nutrient loss occurs during food preparation. These food preparation losses affect how much of each micronutrient will actually be consumed (Johnson, Mannar and Ranum, 2004). Repeated heating, as may be experienced with vegetable oils used for frying, is known to significantly degrade vitamin A (Clarke, 1995). The stability of vitamins and minerals in cooked foods made with fortified maize flour is good. Only vitamin A showed a loss of between 10 and 15% after cooking maize flour for five minutes. According to analyses done in South Africa, the losses of vitamin A during the traditional cooking of maize meal is “somewhat higher” than for maize flour, probably due to the different time-temperature conditions (DSM/USAID, n.d.a). In the final report prepared by the CSIR

on the stability tests and sensory evaluation of fortified food vehicles for the South African National Food Fortification Program it was found that the mean cooking losses of vitamin A after 20 minutes of steaming in super maize meal were 53%, in special maize meal mean cooking losses were 41% and in sifted maize meal 45% (Kuyper, 2000).

Figure 2.8: Examples of maize porridge cooked from white maize meal

2.5.4 Summary

To achieve the required level of nutrients in fortified products reaching the consumer, manufacturers have to estimate processing and storage losses and add the necessary excess during production. To provide the best product to the consumer, the concept of overage should be introduced. Overage is the use of data on nutrient stability to calculate the amount of added nutrient so that the anticipated level of the nutrient at the end of the product's shelf life is in accordance with the level indicated on the package (Wirakartakusumah and Hariyadi, 1998). The introduction of new processes, equipment and packaging materials can affect processing and storage losses and hence fortification procedures (Clarke, 1995).

It would be feasible to add vitamin A to any kind of flour or maize meal. The primary constraint is the cost. Inclusion of vitamin A can double or triple the cost of a cereal fortification program. Vegetable oil may be a better carrier because the form of vitamin A that can be used in oil is cheaper and the stability is somewhat better. However, in many countries, wheat flour or maize meal may be the only processed foods consumed widely.

2.6 Sampling

Food sampling concerns the selection of the individual units of food, food products or bulk foodstuffs from the food supply or source, whether it be from the land, market place, manufacturing/food outlet or from the homes of the members of the study population (field sample). One of the main objectives of food sampling is to provide representative mean values for individual components (nutrients) in foods (Greenfield and Southgate, 2003).

The sampling procedure depends on the aim of the study, e.g. should the sample be representative for the whole country or only for a specific area or project or should the sample cover different seasons or be collected during one growing season. Sample units should preferably be randomly selected.

The following points highlight the important aspects of the sampling procedure.

- Where is the food consumed and by how many?
- How is the food consumed – raw or cooked?

- Are market statistics available? This provides information on the importance of the foodstuff in the food chain. Determine and collect the most used foods/recipes/cooking methods per region/sampling area.
- The population (total amount) of food items may be supplied to or distributed through an entire nation or region or be only typical of a particular sub-population group (e.g. ethnic group or tribe) (SAFOODS, 2010).

A sample is a single unit or a collection of units (e.g. packages, bunches, number of roots, fruits or items) representative of the total population of the food. Sample units must be taken from the available types and forms of the food for which the nutrient composition estimates are being determined. Most sampling schemes adopt a standard of at least 10 food sample units. However, available funds are often a limiting factor in the number of samples that can be analysed (SAFOODS, 2010).

Proper handling and transport of the samples is important to prevent nutrient losses. The samples must be properly identified and described. The sell by dates or batch numbers, the time and date of collection, and the location of collection should be reported. Samples must be packed in suitable containers; especially those that need refrigeration to avoid loss or damage to the food product, particularly of moisture loss or dark containers to avoid vitamin losses. Detailed guidelines on sampling procedures and the handling of the samples must be provided to the person responsible for the collection of the sample (Greenfield and Southgate, 2003).

In this project, convenience sampling was used to sample the major maize meal brands determined by market share and shelfe space. Maize meal samples were selected randomly from shelves in retail stores and outlets of varying size. Immediately after purchase, samples were transported directly to the laboratory in containers that protected them against direct light and heat.

2.7 Measuring the vitamin A content of South African fortified white maize

Development or selection of analytical procedures should be based on consideration of accuracy and precision of measurements, available facilities and equipment, simplicity of procedure and rapidity of determination. Only internationally recognised methodologies should be used (Clarke, 1995). A number of points impact on the suitability of various methods for the determination of vitamin A. These factors include:

- size of the test portion,
- efficiency of extraction procedures,
- chromatographic techniques, and
- adequacy of method validation (Blake, 2007).

2.7.1 Size of the test portion

For the purpose of testing vitamins A, E and β-carotene the European Committee for Standardisation (CEN, 2000) and the AOAC International (AOAC, 2006) state that test portions of a wide range of food products varying in weight between 5 and 10g should be used.

2.7.2 Extraction procedures

Extractions are usually made either by saponification/solvent extraction or by direct solvent extraction. Supercritical fluid extraction has been reported as an alternative, but has not yet been officially accepted (Blake, 2007). Saponification is commonly

used to liberate bound or esterified forms of the vitamin. Saponification is generally performed under reflux conditions with additions of antioxidants such as ascorbic acid or butylated hydroxytoluene (BHT) together with nitrogen flushing to reduce oxidation losses (Hulshof, 2005). After saponification, a liquid-liquid extraction (LLE) step, with non-polar organic solvents is performed. The organic phases are pooled, evaporated to dryness and redissolved in the liquid chromatography (LC) mobile phase. Solid phase extraction (SPE) as an alternative to LLE can also be used, but needs further evaluation (Blake, 2007).

2.7.3 Chromatography

Several techniques have been used for analysis including liquid chromatography (LC), gas chromatography (GC), spectrophotometry and capillary electrophoresis (CE). However the most widely used and preferred method is LC with UV detection (Greenfield and Southgate, 2003; Blake, 2007). The official methods recommend either reverse phase C18 (RP-C18) or straight phase C18 columns (AOAC, 2006; CEN, 2000). The calibration standards for vitamin A must be checked for purity by spectrophotometric procedure and a correction applied (Hulshof, 2005).

2.7.4 Method validation

Method validation is the process of proving that an analytical method is acceptable for its intended purpose. Many analysts focus on validating a method for precision, limit of detection (LOD), limit of quantification (LOQ), linearity and range (Green, 1996). The use of inter-laboratory studies and reference material is a prerequisite for checking correct application of analytical methods and to check accuracy (Blake, 2007).

In this study a method was optimised and validated to determine vitamin A in maize meal, maize porridge and liver samples. Alkaline saponification of the test material to eliminate fats, liberate natural retinol in the cells and hydrolyse added vitamin A to retinol was used. This was followed by ether extraction of unsaponifiable material. Quantification was done by HPLC and photo diode array (PDA) detection. The concentrations of the standards were calculated by using the Beer-Lambert Law.

2.8 Studying the relative bioavailability of vitamin A in fortified maize meal

Measuring the change in serum retinol concentrations following intervention can be used to determine the relative bioavailability of vitamin A, but numerous factors affect results from this approach. The vitamin A in the blood is tightly regulated and dependent on vitamin A status and the amount administered in the dose or meal (Van Lieshout et al., 2001). Other methods to evaluate bioavailability include postprandial chylomicron response (Parker et al. 1999), Caco-2 cells as an in vitro model of the human small intestinal mucosa to predict absorption (Garrett, Failla and Sarama, 1999; Liu, Glahn and Liu, 2004), stable isotope tracers (Vitamin A Tracer Task Force, 2004), and animal models (Baker, 2008). Results from the postprandial chylomicron response model are highly variable among subjects, limiting their use (Parker et al.; 1999). Caco-2 cells investigate bioaccessibility at the intestinal level, but do not reflect influences by the liver or other organs regulating enzyme activity and altering conversion factors. Isotope tracer studies in human hosts are the best method (Vitamin A Tracer Task Force, 2004), but their expense is limiting and factors such as diet and vitamin A status are difficult to control.

Although it would be ideal to use human subjects directly to answer this critical question regarding vitamin A availability, it was not possible because of the finite financial scope of the project. Animal research has contributed a great deal to what we know today about nutrition and metabolism. Appropriate animal models may contribute to a better understanding of vitamin A absorption (Baker, 2008). Animals also have the advantage of allowing invasive tissue sampling to assess nutrient status. Monitoring compliance with dietary protocols is easier with animals. Other considerations include availability of facilities and cost of the experiments to be performed.

2.8.1 Animal models in nutrition research

Animal models were instrumental in solving vitamin deficiency diseases. In his article on animals in nutrition research Baker (2008) discusses a list of examples, such as:

- beri-beri (chick thiamine deficiency),
- scurvy (guinea pig ascorbic acid deficiency),
- pellagra (dog, rat, pig, and chick deficiency of niacin and tryptophan),
- rickets (dog, rat, and chick deficiency of Ca, P, and/or vitamin D),
- night blindness (rat deficiency of vitamin A),
- dermatitis (rat deficiency of vitamin B_6),
- low fertility and muscle dystrophy (rat deficiency of vitamin E),
- haemorrhagic disease (chick deficiency of vitamin K), and
- anaemia (monkey, rat, and chick deficiency of folate and/or vitamin B_{12}).

Many of these diseases were initially thought to be of infectious and of bacteriologic origin. However, when an association with diet was noted, and when this was

followed by development of animal-model bioassays with defined purified diets, progress was quickly made in defining the disease condition and in reversing or preventing it with the proper vitamin containing food or (later) with the vitamin itself.

Chickens were selected as an appropriate animal model for this study because they are manageable, affordable and most importantly the metabolism of vitamin A in chickens is closely related to that of humans. Vitamin A is stored in the liver and chickens are also very susceptible to vitamin A deficiencies with symptoms very similar to human subjects (NRC, 1994). Chickens respond more rapidly to vitamin deficiencies than pigs (Baker, 2008). Therefore an effect caused by different diets will become evident over a shorter period of time. Significant results are most likely to be obtained in a study using chickens to determine the relative bioavailability of the vitamin A fortificant in fortified maize meal when comparing different maize meal diets with each other.

2.9 Concluding Remarks

Vitamin A deficiency is a public health concern in South Africa as is he case in many low income countries particularly in preschool-aged and school-aged children, as well as women of reproductive age. Fortification of foods with vitamin A is a potentially effective food-based intervention to prevent or control vitamin A deficiency in low-income countries where undernutrition and poverty coexist. According to the literature survey, maize meal is a suitable vehicle for vitamin A fortification. Fortification should be guided by estimates of intakes of vitamin A in the diet, levels of fortificant required to meet dietary requirements, stability of the fortificant under ambient conditions, stability under usual conditions of food preparation (e.g., high temperature and humidity) and product storage conditions.

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CHAPTER 3

VITAMIN A CONTENT IN FORTIFIED WHITE MAIZE MEAL AS PURCHASED AND IN PORRIDGE AS CONSUMED IN SOUTH AFRICA

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A method to determine vitamin A in maize meal was optimised and validated. The method was accredited by the South African National Accreditation Services (SANAS). This method was subsequently used to determine the vitamin A content of maize meal samples, as well as the corresponding maize porridge samples. Retention of vitamin A in cooked porridge was calculated.

3.1 Abstract

In 2003, Department of Health of South Africa embarked on a mandatory fortification program of maize meal as part of a nutrition program to alleviate malnutrition. The aim of this study was to determine the vitamin A content in fortified white maize meal and the maize porridge prepared with it as purchased and consumed. The highest mean vitamin A concentration in the maize meal was 261 µgRE/100g and the lowest mean vitamin A concentration was <19 µgRE/100g. Pertaining to regulation the final minimum level of vitamin A in fortified maize meal shall not be less than 187.7 µgRE/100g (Department of Health, 2003). The average retention of vitamin A in maize porridge as the difference in vitamin A concentration between raw maize meal

and cooked porridge was calculated as 39.8%. Although fortification of maize meal can improve the vitamin A intake of the population, it must be regularly monitored and regulated to be beneficial. If not then fortification might as well be voluntary.

Key words: maize meal, porridge, vitamin A fortification, retention, staple foods

3.2 Introduction

Food fortification of staple foods with micronutrients is one of the food-based strategies employed to alleviate micronutrient deficiencies in a population. Vitamin A deficiency (VAD) is a major nutritional concern in poor societies, especially in lower income countries. Its presence as a public health problem is assessed by measuring the prevalence of deficiency in a population, represented by specific biochemical and clinical indicators of status (WHO, 2009a).

In South Africa, 1 in 3 preschool children has a serum retinol concentration < 0.7 µmol/L (SAVACG, 1996), and 55–68% of children aged 1–9 years consume < 50% of the recommended dietary intake of vitamin A (700 µg retinol equivalents) (NFCS, 2000). The main underlying cause of VAD as a public health problem is a diet that is chronically insufficient in bioavailable vitamin A that can lead to lower body stores and fail to meet physiologic needs (e.g. support tissue growth, normal metabolism, resistance to infection) (WHO, 2009a).

In 2003, the Department of Health of South Africa embarked on a fortification program of wheat flour and white maize meal as part of a multipronged approach to alleviate malnutrition. These foods were identified during the National Food Consumption

Survey (NFCS, 2000) as most often consumed (staple) food products, thereby reaching lower income consumers most vulnerable to micronutrient malnutrition. According to regulations protected, stabilized Vitamin A palmitate containing 75 000 µRE activity per gram premix must be added to the maize meal (special, super, sifted and unsifted) to give a final, minimum level of the micronutrient in the fortified maize meal of 187.7 µRE activity per 100 g (Department of Health, 2003).

The success of a fortification program depends, amongst other factors, on the content of the fortificants in the fortified products. A number of factors, including nutrient interactions, the stability of the specific micronutrients added to the food under anticipated conditions of storage and processing can all have an influence on the fortificant concentration. The choice of a vitamin A fortificant is largely governed by the characteristics of the food vehicle. Because preformed vitamin A (retinol) is an unstable compound, in commercial preparations it is esterified, usually with palmitic or acetic acid, to the more stable corresponding esters. Retinyl acetate and retinyl palmitate are the main commercial forms of vitamin A that are available for use as food fortificants in cereals (WHO, 2006). Maize meal can technically be fortified with vitamin A because vitamin A is stable in dry products without producing organoleptic changes. Vitamin A is quite stable when heated to moderate temperatures in the absence of oxygen and light. However, as is the case for some other vitamins, high humidity, high temperatures and the presence of oxygen and light can adversely affect the vitamin A content during the preparation of maize meal products such as traditional maize porridge (or “pap”). This reaction is also accelerated in the presence of trace metals (Mehansho et al., 2003; WHO, 2009b).

It would thus be feasible to add vitamin A to any kind of maize meal with the primary constraint being cost. Inclusion of an expensive micronutrient such as vitamin A can double or triple the cost of a cereal fortification program due to the cost of the

micronutrient, extra equipment needed for mixing, quality control through quantitative vitamin analysis and additional personnel (WHO, 2009b).

An early quality control step to make sure that the food fortification program will have an impact on vitamin A deficiency is to verify the vitamin A content in the fortified maize meal as well as in the cooked products. If these comply with regulations, a reduction in vitamin A deficiency can be assumed in the long term.

Therefore the aim of this study was to determine the vitamin A content of fortified white maize meal from different manufacturers (brands) as purchased from the shelves of different retailers, as well as in the traditional maize porridge as consumed. Due to financial constraints and the fact that the vitamin A fortificant is stable under dry storage conditions (WHO, 2009b), a shelf-life study was not done.

3.3 Materials and Methods

Note: Light should be avoided during preparation and storage of samples and standards to prevent degradation of vitamin A.

3.3.1 Samples

Sixty-two samples of fortified white maize meal from readily available brands (nine different brands) were collected from supermarkets in the Tshwane-metropolis between July 2005 and November 2008. See Addendum B for a list of brands. Samples were stored in their original packaging at room temperature in the laboratory until analysis. Analyses commenced within a week after every sampling. Brands with a higher market

share according to the Markinor/Sunday times Top Brands Results (2008) have higher representation within the data set. A wide variety of maize porridge preparation methods is known, but due to resource constrains only preparation of the traditional soft porridge was selected for laboratory simulation. The maize porridge was prepared according to a standardised method from seven different brands of maize meal purchased from the supermarkets. Each maize meal and its corresponding porridge sample were analysed in duplicate for moisture and vitamin A content using accredited methods according to ISO/IEC 17025:2005. The methods were accredited by the South African National Accreditation System (SANAS).

Figure 3.1: Different maize meal brands sampled during the study.

3.3.2 Preparation of porridge samples

Traditional soft maize porridge was prepared according to the following recipe: One litre (1L) of tap water was heated to boiling point in an aluminium saucepan. A 180 g sample of dry maize meal was added and stirred thoroughly. The heat was turned down and the porridge was left to simmer with the lid on for 30 minutes, whilst stirring

occasionally. The end-temperature of the samples was between 75 ℃ – 80 ℃. The samples were prepared with the assistance of people familiar with the preparation method, texture and consistency of this type of traditional porridge. Porridge samples were left to cool in covered glass containers and were stored under refrigeration (± 4℃) until the next day when they were analysed.

3.3.3 Gravimetric determination of dry matter

Dry matter was measured in the samples by determining the loss in weight of the sample after it had been dried in an oven at 105±1℃ for 16 hours. Weight loss is used to calculate dry matter content (AOAC, 2005a).

3.3.4 Determination of total Vitamin A as all-trans retinol

3.3.4.1 Chemicals and Standards

Diethyl ether, ethanol (99.9%), potassium hydroxide (KOH) and sodium chloride (NaCl) were obtained from Merck Chemicals. Butylated hydroxyl toluene (BHT) and retinol standard were purchased from Sigma-Aldrich. HPLC-grade methanol was obtained from Labscan and pure, crystallised ascorbic acid from Associated Chemical Enterprises. A stock standard solution of retinol was prepared in ethanol. Working standard solutions were prepared in ethanol and the concentration of each standard was determined with a spectrophotometer (Lambda 25, PerkinElmer), using an extinction coefficient of 1850 (λ_{max} = 325 nm).

3.3.4.2 Sample preparation

Approximately 5 g maize meal or 8 g porridge was weighed into a round bottom flask using a Precisa XT220A analytical balance (readability ± 0.1mg).

3.3.4.3 Saponification

The weighed sample was mixed with 25ml of a 0.5% ascorbic acid-ethanol-methanol solution until sample material was moistened. Glass beads were added and purged with nitrogen gas. The sample was saponified at boiling point for 30 min under reflux with 50% KOH (w/w). The flask was swirled from intermittently to prevent the material from adhering to the sides. After saponification the sample was cooled on ice for 5 minutes.

3.3.4.4 Extraction and phase transfer

The contents of the round bottom flask were filtered through Whatman no 4 filter paper into a separating funnel. The flask was rinsed with a minimum amount of water (no more than 15 – 30ml) and filtered into the separating funnel. Subsequently the round bottom flask was washed with diethyl ether containing 0.01% BHT and added to the separating funnel. The mixture was allowed to expand several times before the actual extraction (in such conditions emulsions can be largely avoided). The ether layer was decanted into another separating funnel. Extraction was repeated two more times combining all the ether fractions in the same separating funnel. The ether fraction was washed with distilled water until neutral. Should any emulsions form during the wash and extraction procedures, NaCl can be added. The ether fraction was then transferred to a 250ml volumetric flask and made up to volume with diethyl ether. An aliquot from

the ether extract was evaporated to dryness with a rotary evaporator under partial vacuum in a water bath at a temperature < 40°C. The residue was dissolved in ethanol and injected into the HPLC.

3.3.4.5 HPLC

The HPLC system (Shimadzu) consisted of a Quaternary gradient pump (model LC-20AD), a solvent degasser (model DGU-20A5), an auto-injector (model SIL-20A, 230V), a Photodiode Array Detector (DAD) with a thermostatted standard cell (model SPD-M20A) and control and integration software (LCsolution Ver. 1.1). A Nucleodur 250X4 mm reverse phase C18 column (5µm particle size) with guard column was used. Separations were achieved using a mobile phase of 97% methanol in deionised water and a flow rate of 1.0 mL/min. Separations were performed at 325 nm for the identification and quantification of retinol.

3.3.4.6 Calculation

Quantification was performed by using an external calibration procedure. The peak height of five different concentrations of a retinol standard and a blank (ethanol) were used for calibration. The calibration standards were checked for purity and concentration by spectrophotometric procedure.

3.3.4.7 Method validation

Retinol was determined by using peak height and regression analysis. From the calibration curve, linearity, range, limit of quantification (LOQ) and limit of detection (LOD) were determined. The LOQ and LOD were calculated from the calibration lines

that defined linearity, using the Long and Winefordner criterion (Long and Winefordner, 1983) as expressed in the following equations.

$$LOQ = \frac{10 \times S}{a}$$

$$LOD = \frac{3 \times S}{a}$$

where *a* is the slope of the calibration line and *S* is the standard error of the intercepted point. The LOQ, LOD and precision of the method are shown in Table 3.1.

Repeatability of the method was determined by analysing the same sample eight times on the same day. From this data the mean, standard deviation (SD) and coefficient of variation (CV%) were determined. Reproducibility of the method was determined by analysing a control sample (infant cereal with added vitamins) over a period of time (≥ 7 times). The mean, standard deviation and coefficient of variation were calculated. A control chart was implemented to monitor validity of the analysis. The action limits were set as the mean plus or minus three times the standard deviation of the reproducibility data. Warning limits were set as twice the standard deviation. The control sample (infant cereal) was analysed with every batch of ten samples or less. The results of the control sample were recorded on the control chart and evaluated. If the result fell outside the action limits, the analysis was repeated. Standard reference material (SRM2383 – baby food composite) and inter-laboratory comparisons (using fortified maize meal as a control sample) were used to prove accuracy.

3.3.5 Calculation of the retention of vitamin A in porridge

Retention of vitamin A was calculated based on the following equation (Bengtsson et al., 2008):

$$\%\ \ \mathrm{Re}tention = \frac{retinol\ \ content\ \ per\ \ g\ \ porridge\ \ (dry\ \ basis)}{retinol\ \ content\ \ per\ \ g\ \ meal\ \ (dry\ \ basis)} \times 100$$

The vitamin A result of each maize porridge sample was compared with its corresponding maize meal sample.

3.3.6 Statistical Analysis

Data was analyzed by Analysis of Variance (ANOVA), Pearson's correlation test and Principal Component Analysis (PCA), which were applied to determine and explain variation in the data. The data was analysed with SAS statistical software version 9.2 (SAS, 1999).

3.4 Results and Discussion

3.4.1 Method performance

Blake (2007) evaluated several official AOAC, CEN and ISO methods for the determination of fat soluble vitamins. These methods involve alkaline saponification of the test material to eliminate the fat without removing the fat-soluble vitamins, liberate natural retinol in the cells and to hydrolyse added vitamin A in fortified food products to retinol. After saponification, the vitamins are separated by liquid-liquid extractions with organic solvents. The organic phases are pooled and evaporated to dryness. This is then redissolved in the mobile phase and usually analysed by liquid chromatography.

Table 3.1: Limit of Detection (LOD), Limit of Quantification (LOQ) and Precision

	LOQ	LOD	Repeatability			Reproducibility		
	(µg/100g)	(µg/100g)	Mean	SD	CV%	Mean	SD	CV%
All-*trans* Vitamin A	20	7	0.536	0.057	10.593	0.588	0.082	14.017

The performance of the method was determined as summarised in Table 3.1. Precision was assessed using the criteria developed by AOAC International (2005b). The calculated Horwitz Ratio (HorRat) of 1.28 for repeatability and 1.13 for reproducibility is consistent with the guideline range of 0.5 – 2.0. Linearity was confirmed by least-squares regression analysis of the calibration standards. The UV-signals (peak height) were linear in the range 0 - 566 µg/100ml with an accepted linearity of $R^2 \geq 0.98$. Accuracy was determined by two different inter-laboratory studies as well as with a standard reference material. The values were compared and acceptable z-scores (<2) obtained. The method was validated for linearity, repeatability, reproducibility, LOD, LOQ and accuracy.

3.4.2 Vitamin A content of maize meal as purchased in supermarkets

Vitamin A concentrations were evaluated for outliers using the Q-test. Outliers were excluded from the data set. The mean vitamin A content per brand can be seen in Figure 3.2. Brand A had the highest mean vitamin A concentration (261 µgRE/100g), and is also the only brand analysed with a higher mean vitamin A concentration than the regulatory requirement of 187.7 µgRE/100g (Department of Health, 2003). Brand D had the lowest mean vitamin A concentration (<19 µgRE/100g).

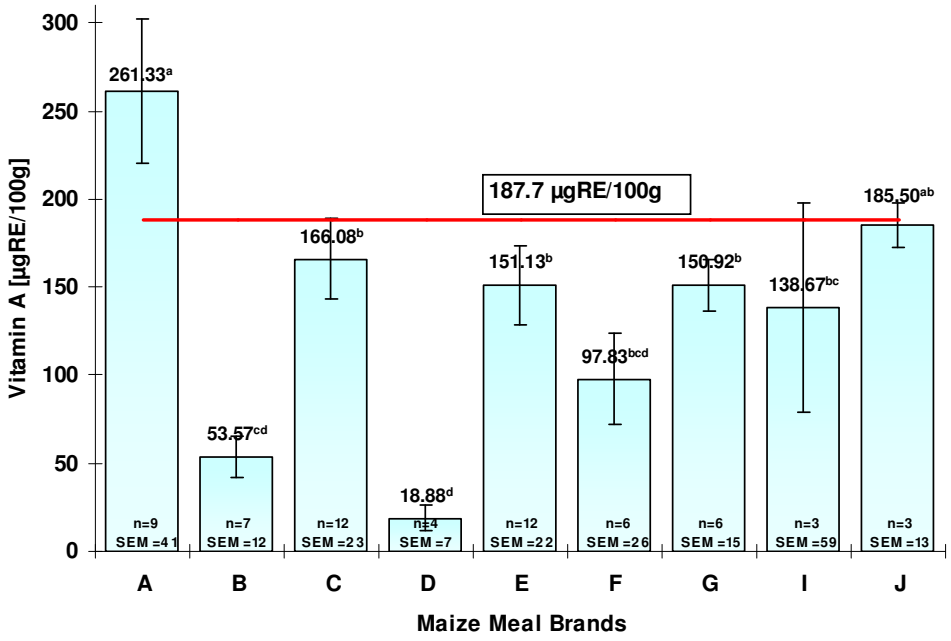

Figure 3.2: Mean vitamin A concentration (µgRE/100g) of different brands of maize meal as purchased in supermarkets in the Tshwane-metropolis

According to fortification principles, the maize meal is fortified with protected and stabilised Vitamin A palmitate to improve stability of the added vitamin. The protected particles tend to be heterogeneously distributed throughout the maize meal, and this may influence the precision of the analyses (Blake, 2007). This may also cause segregation of the maize meal leading to a variation of vitamin A content within one brand of maize meal. This could explain the large variation in results within a specific maize meal brand.

Although there is a regulatory requirement for vitamin A, a large variation in vitamin A content between different brands was observed. This variation may be an indication of poor quality control at the millers. Poor or variable quality of fortification premixes, unreliable and poorly fabricated equipment, and inadequate manufacturing and marketing facilities lead to poor product quality (Johnson, Mannar and Ranum; 2004).

Another reason for the low vitamin A concentration in the maize meal might be the incorrect storage conditions of the maize meal on the shelves of retailers. It was observed during the sampling of the maize meal that some of the maize meal was exposed to sunlight. As was previously mentioned, vitamin A is light sensitive (DSM/USAID, n.d.b). If the maize meal remains on the shelves for several days, it may have an effect on the vitamin A content.

Fortification mixes supplied by unregistered suppliers and the stability of the vitamin A are challenges identified by the Department of Health of South Africa (de Hoop; 2010). Major obstacles to the implementation of an adequate food control system (FCS) occur when material sourcing, production, packaging, storage, transport conditions and delivery systems are sub-optimal. The lack of efficient and skilled manpower to carry out an effective FCS both at production and government levels, coupled with limited training opportunities are other major obstacles (Clarke, 1995). Moreover, legislation and regulation in South Africa may not be well developed. Enforcement mechanisms are probably not yet adequately developed and established to ensure that government standards are met.

3.4.3 Vitamin A concentration of maize porridge

Vitamin A and dry matter content were determined for each of the maize meal samples and the corresponding porridge samples. An average retention of 39.8% was observed. Results are shown in Table 3.2.

Table 3.2: Vitamin A content (µgRE/100g dry matter) of maize meal and maize porridge samples of seven different brands

Brands	Maize Meal (Raw)	Maize Porridge (Cooked)	% Retention of Vitamin A
	*Vitamin A (µgRE/ 100g DM)		
A	174.7	85.5	48.9
B	93.7	45.5	48.6
C	238.8	83.9	35.1
D	10.5	5.90	55.8
E	237.8	45.3	19.0
G	245.4	90.5	36.9
J	201.6	69.1	34.3
		Average retention	**39.8±12.3**

*Vitamin values are reported on a dry weight basis.

The average cooking losses of vitamin A in super maize meal according to the CSIR-report on the stability of fortified food vehicles for the National Food Fortification Program was reported as 53% (Kuyper, 2000). This relates to an average retention of 47%. When the more recent nutrient composition values of super maize meal, as reported by Wolmarans, Danster and Chetty (2005) were used, retention of 39.5% was calculated for soft porridge, which compared favourably with results of this study. The result is best explained by the fact that vitamin A is stable under inert atmosphere. However, it rapidly loses its activity when heated in the presence of oxygen (Lešková et al., 2006).

A Pearson's correlation test (Table 3.3) and principal component analysis (PCA) were done to determine whether there was an association between the retinol concentration and dry matter (DM) in the maize meal (raw) and the retinol concentration and dry matter in the maize porridge (cooked). The correlation between retinol and dry matter in the raw maize meal is not significant ($r = -0.525$; $p > 0.05$). This is expected as retinol is not related or dependant on the dry matter content. The correlation between the dry matter in the raw maize meal and in the cooked porridge is significant ($r = -0.542$; $p \leq 0.046$). Although the dry matter contents in the raw maize meal and in cooked maize meal (porridge) are dependant on each other, it must be taken into account that the matrix of the maize meal changes during cooking because water is absorbed and heating causes starch gelatinisation. As is expected, the correlation between the retinol in the maize meal and in the maize porridge is high ($r = 0.833$; $p \leq 0.000$), but not identical. This is supported by the retention values calculated and is an important consideration in determining fortification levels.

Table 3.3: Pearson Correlation matrix between retinol content and dry matter (DM) of the raw maize meal and retinol content and dry matter (DM) of the cooked maize porridge

Variables	Raw-DM	Raw-Retinol	Cooked-DM	Cooked-Retinol
Raw-DM		-0.525 (p>0.054)	-0.542* (p≤0.046)	-0.576* (p≤0.031)
Raw-Retinol			0.087 (p>0.767)	0.833*** (p≤0.000)
Cooked-DM				-0.038 (p>0.897)

Significant levels: * p≤0.05; ** p≤0.01; *** p≤0.001)

The PCA explained 77.71% of the variation in the data. See Figure 3.3 for the biplot of retinol and dry matter (DM) in maize meal (raw) and maize porridge (cooked). On PCA1 (x-axis) 50.94% of the data was explained. The variables retinol-raw (31.38%), DM-raw (-25.89%) and retinol-cooked (23.98%) contributed the most to the variation. On PCA2 (y-axis) 26.77% of the data was explained by the variables DM-cooked (46.68%) and retinol-cooked (25.57%). If the retinol value in the raw and cooked samples were high then the dry matter was low.

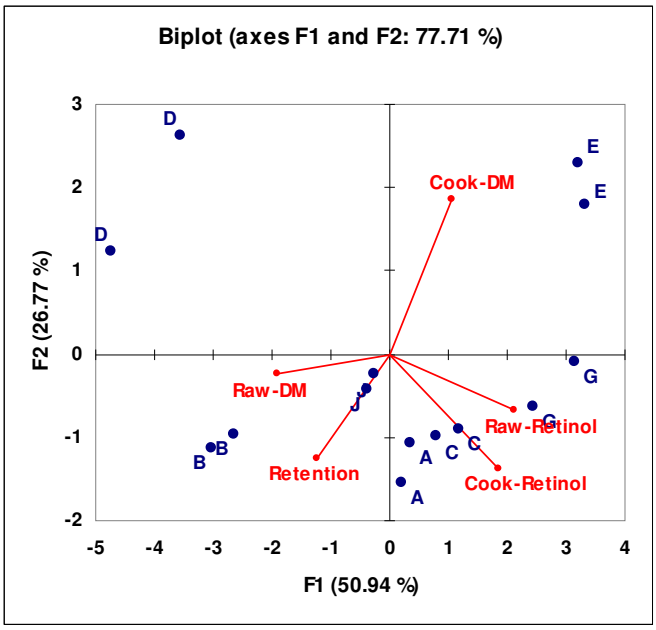

Figure 3.3: PCA Biplot of retinol and dry matter (DM) in maize meal (raw) and maize porridge (cooked)

To understand the contribution of the fortified maize meal to the vitamin A intake of children, the results of the different brands were translated (see Table 3.4) into Recommended Dietary Allowance (RDA), Daily Recommended Intake (DRI) and Recommended Safe Intake (RSI) values (FAO; 2001) . The RDA and DRI for children

1-3 years and 4-9 years is 300 and 400 µg retinol/day respectively. The RSI values used by the FAO to correct VAD in a population are 400, 450 and 500 µg retinol/day for children 1-3 years, 4-6 years and 7-9 years respectively. According to the National Food Consumption Survey (NFCS, 2000) the average portion size of maize porridge reported for children 1-3 years was 410 g/person/day and for children 7-9 years was 500 g/person/day. This relates to an average portion size of 455 g/person/day for children 1-9 years (12-108 months). This portion size was used in the calculation of the average intake of vitamin A from soft maize porridge based on the concentration levels as determined in this study.

The highest contribution to the RDA and RSI was made by maize meal Brand G and the lowest contribution by Brand D. On average, 17% of Recommended Dietary Allowance (RDA) for children 1-3 years and 13% of RDA for children 4-9 years old were met by the fortification of the maize meal (Table 3.4). It must be kept in mind that this would be a zero percentage if the maize meal was not fortified, but that it should be a 31% of RDA according to legislation. This contribution is even lower when compared to at the Recommended Safe Intake levels using by the FAO. When using the same retention values as calculated in this study, the contributions to the RDA for children from maize meal that is fortified according to the minimum levels as stipulated in the regulations (ie. 187.7 µg vitamin A/100g), will only be 16% and 12% respectively. This is approximately half of the 31% government intended to at least achieve through the mandatory fortification of maize meal (Department of Health, 2003).

Table 3.4: Vitamin A content (µgRE/100 g) of maize meal and maize porridge samples of seven different brands and the contribution towards the Recommended Daily Allowances (RDAs) and Recommended Safe Intake (RSI) of vitamin A for 1-9 year old children

Brands	Maize Meal	Maize Porridge	Vitamin A/portion size*	% RDA#	% RDA#	% RSI$	% RSI$	% RSI$
	Vitamin A (µgRE/100g)		(µgRE/100g)	(1-3 years)	(4-9 years)	(1-3 years)	(4-6 years)	(7-9 years)
A	155	16	71	24	18	18	17	14
B	83	8	34	11	8	8	7	7
C	212	15	67	22	17	17	15	13
D	9	1	5	2	1	1	1	1
E	210	9	42	14	10	10	9	8
G	217	18	80	27	20	20	18	16
J	180	13	56	19	14	14	12	11
			Average contribution	**17±9**	**13±6**	**13±6**	**11±6**	**10±5**

* Portion size: 445 g/person/day for maize porridge (NFSC, 2000)

#The RDA and DRI of vitamin A for children 1-3 years and 4-9 years is 300 and 400 µg vitamin A/day respectively.

$The RSI values of vitamin A are 400, 450 and 500°µg vitamin A/day for children 1-3 years, 4-6 years and 7-9 years respectively.

In essence, food fortification can contribute to the improvement of the overall vitamin A status of children aged 1– 9 years. This was also reported by Steyn, Nel and Labadarios (2008) in their analysis of dietary micronutrient intake pre- and post-fortification using existing dietary data. However, it is suggested that the level of vitamin A fortification be raised to at least achieve the intended 31% RDA contribution or even higher. A review by Allen and Haskell (2002) indicated that the risk of excessive vitamin A consumption from fortified foods in women and young children is likely to be negligible.

3.5 Conclusion

The quantitative difference in the vitamin A content of fortified white maize meal as purchased and consumed is shown. Vitamin A concentrations varied from the highest concentration of 226 µgRE/100g to the lowest concentration of <19 µgRE/100g. Reasons for the large variation in vitamin A concentration could be explained by substandard premixes, inadequate mixing of the fortification premix into the maize meal, segregation of the fortificant and the maize meal, storage losses or poor quality control by the milling companies. The average retention of vitamin A in maize porridge was calculated as 39.8%. The low retention observed might be an indication of poor stability of the vitamin A fortificant under cooking conditions.

The lower than regulated concentration levels and the low retention of the vitamin A found in this study, probably contribute towards the RDA for vitamin A for 1-9 year old children not being met. This may explain the results found during the National Food Consumption Survey Fortification Baseline Study (NFSC-FB-I) done in 2005. One of the main findings in this study was that the prevalence of poor vitamin A status in

children appeared to have increased when compared with previous national data (NFSC-FB-I; 2008). This emphasises the need for an efficient food control system (FCS) in South Africa in order that food fortification processes meet nutritional objectives. Evaluation of some of the more mature fortification programs, mainly in Latin America, suggests that the quality of vitamins, minerals and micronutrient premixes may be a barrier to achieving the required health and nutrition results (DSM; 2009). A consideration that should be high on the priority list of the overall micronutrient strategy is the adequate and efficient monitoring, evaluation, regulation and quality assurance of all premixes and maize meal.

Correcting VAD in populations at risk of deficiency is an investment in improving human development. Based on the results and evaluation of this study, it appears that fortification of maize meal can contribute to the micronutrient intake of children under nine years of age and improve the overall micronutrient density of their diets. It is necessary to take into account whether the efficacious nutrient supply can be met. The efficacious nutrient supply depends on the amount of vitamin A-containing foods consumed, vitamin A content of each food consumed and bioefficacy of vitamin A in the food consumed (Van Lieshout and West, 2004). It is therefore important to also verify the vitamin A concentration in bread as this is the other food vehicle used for fortification and to evaluate the bioavailability of the added vitamin A.

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CHAPTER 4

EFFECT OF DIFFERENT MAIZE MEAL DIETS ON THE GROWTH AND VITAMIN A STATUS OF CHICKENS

The relative efficacy of the daily consumption of fortified maize meal in sustaining or improving vitamin A status was evaluated. Although children could be used to evaluate their vitamin A status after consumption of fortified maize meal, this was beyond the financial means of the project and such an approach also has limitations. Consequently, chickens were used as the biological model. Growth and vitamin A status were evaluated using the weight, feed conversion and liver retinol stores of the chickens on different diets over a six week period.

4.1 INTRODUCTION

Vitamin A deficiency (VAD) is reported as being the nutritional health problem of highest public health significance in developing countries after protein-energy malnutrition and iron deficiency anaemia (Ahmed and Darnton-Hill, 2004). In South Africa, 1 in 3 preschool children has a serum retinol concentration <0.7 µmol/L (SAVACG, 1996) and 55–68% of children aged 1–9 years consume <50% of the recommended dietary intake of vitamin A (700 µg retinol equivalents) (NFCS, 2000). Children living in rural areas are the most affected by VAD (SAVACG, 1996; NFCS, 2000). VAD is mainly caused by a diet that provides too little vitamin A to meet physiological needs.

Maize is the most important grain crop in South Africa given its status as a staple food product for more than 50% of the population and its central role in feed formulations. The National Food Consumption Survey (NFCS, 2000) identified refined white maize meal as currently the main staple food for human consumption in South Africa while yellow maize is preferred for animal feeds and manufacturing of breakfast cereals and snacks (Graham and Rosser, 2000). White maize meal is however, refined to such an extent to meet consumer preferences that it is little more than pure starch. This final product unfortunately primarily contributes energy to the diet and very little protein and essential vitamins and minerals. The Department of Health of South Africa embarked on mandatory fortification of wheat flour and maize meal with vitamin A, iron, zinc, folic acid, thiamine, niacin, vitamin B6 and riboflavin since October 2003 as part of a multi-faceted approach to alleviate malnutrition (Department of Health, 2003). Two of the considerations in a fortification program are the availability and absorption of the added micronutrients in the fortified foods.

Regarding vitamin A absorption it would be ideal to use human subjects to answer this critical question. However, this was not possible within the financial scope of this project. Appropriate animal models on the other hand may contribute to a better understanding of vitamin A availability and vitamin A absorption. An ideal model should have the following characteristics: *1*) demonstrate absorption of the vitamin which will be intact at physiological levels, similar to humans; *2*) reflect a distribution of vitamin A in tissues and serum similar to that of humans; *3*) be representative of the disease state of interest; *4*) be readily available; *5*) be easily manageable in a laboratory setting; and *6*) be affordable.

Unfortunately, no one model meets all of these criteria (Lee, et al.; 1999). Chickens were selected as the animal model used in this study, as they are manageable, affordable and most importantly the metabolism of vitamin A and carotenoids in

chickens is closely related to that of humans. Chickens are also very susceptible to vitamin A deficiencies with symptoms very similar to humans and significant results are most likely to be obtained (NRC, 1994).

The aim of this study was to determine the relative efficacy of the daily consumption of fortified maize meal in sustaining or improving the vitamin A status, by using a chicken model. Growth and vitamin A status were evaluated by the weight, feed conversion ratio and liver retinol stores of the chickens on different diets over a six week period.

4.2 Materials and Methods

4.2.1 Husbandry and rearing of broilers

The experiment was conducted at the Poultry Nutrition Facility of the ARC: API, Irene, South Africa. The protocol was approved by the ARC-Irene Animal Ethics Committee (Ref no: APIEC07/01) (Addendum B). Day-old broilers (Ross 788) were obtained from a commercial hatchery. Upon arrival at the research site, the chicks were examined and only healthy chicks were included in the study. The broilers were placed in a temperature controlled broiler room (maintained at 32±2℃). The vaccination program applied was according to the Poultry Reference Laboratory at the University of Pretoria, Onderstepoort. The trial was conducted until the broilers were 42 days old.

The experiment was designed as a randomized complete block with six replicates per treatment. The diets were formulated according to the specific nutrient composition that is required for broiler starter (week 1-3) and grower (week 4-6) diets, except for the vitamin A source in each sample (Tables 4.1 and 4.2). The fortified white maize meal used (TRM1, TRM2 and TRM3), was purchased at a retail outlet as commercially

available to the consumer. The yellow maize meal (TRM4 and TRM5) is feed grade as commercially available to the poultry industry. The vitamin and mineral premixes with Salinomycin were obtained from Advit Animal Nutrition a company supplying vitamin and mineral premixes for animal nutrition.

Table 4.1: Diet formulation for broiler starter and grower diets (% of total diet)

Treatments	Starter	Grower
Maize meal	60.84	72.92
Sunflower Oil Cake	3.96	*
Soyabean Oil Cake	19.86	12.82
Maize Gluten 60	11.37	10.39
Limestone	2.16	2.24
Salt	0.39	0.25
L Lysine HCL	0.14	0.10
DL Methionine	0.20	0.20
Mono Ca P	0.50	0.50
Vitamin & Minerals	0.50	0.50
Salinomycin	0.05	0.05

Table 4.2: Source of vitamin A per treatment

	Source of Vitamin A	
	Premix	**Maize Meal**
Treatment 1 (TRM1) Fortified white maize meal (Brand F) with normal vitamin and mineral premix optimised for chickens; without vitamin A supplementation	**-**	**X**
Treatment 2 (TRM2) Fortified white maize meal (Brand A) with normal vitamin and mineral premix optimised for chickens; without vitamin A supplementation	**-**	**X**
Treatment 3 (TRM3) Fortified white maize meal (Brand A) with normal vitamin and mineral premix optimised for chickens; with vitamin A supplementation	**X**	**X**
Treatment 4 (TRM4) Yellow maize meal with normal vitamin and mineral premix optimised for chickens; with vitamin A supplementation	**X**	**-**
Treatment 5 (TRM5) Yellow maize meal with normal vitamin and mineral premix optimised for chickens; without vitamin A supplementation	**X**	**-**

A total of 900 broilers were randomly allocated to 30 pens, each containing 30 birds. Each of the five treatments was replicated six times. A total of 60 chickens (two per pen) were randomly selected from every pen for initial sampling of livers to determine the baseline vitamin A concentrations. Chickens were culled humanely using the dislocation of the cervical vertebra technique. Thereafter, two broilers per pen were culled every seven days from day 0 until day 21 (Starter diet) and one broiler per pen was culled, every seven days from day 21 until day 42 (Grower diet). The livers were excised, placed into clearly marked plastic bags and frozen at -20°C. The frozen livers were sent to the laboratory for determination of the vitamin A concentration.

Figure 4.1: Chickens feeding in the different pens during the feeding trial.

4.2.2 Measurements and observations:

4.2.2.1 Birds

Origin and disease status were obtained from the hatchery. Birds were weighed weekly on a per pen basis starting from day 0 until 42 days of age.

4.2.2.2 Feed

Feed samples per treatment were taken weekly and vitamin A was determined in duplicate. Samples were stored under refrigeration (± 4°C) until analysis.

4.2.2.3 Feed conversion ratio

Cumulative feed intake divided by the body weight gain was calculated on the data weekly. The data were corrected for mortality.

4.2.2.4 Mortality

Pens were checked twice daily for mortality. All mortalities were weighed.

4.2.2.5 Livers

All livers were freeze-dried and vitamin A was determined in duplicate. To account for storage losses of vitamin A, liver samples of the same week were analysed at the same time.

4.2.3 Vitamin A analysis

Analysis was performed at the ARC-Irene Analytical Services using a method accredited according to ISO/IEC 17025:2005. The accreditation body is the South African National Accreditation System (SANAS).

4.2.4 Statistical analysis

The data was analysed with SAS statistical software version 9.2 (SAS, 1999). Analysis of variance (ANOVA) was used to test for differences between treatments. The Shapiro-Wilk test was performed to test for normality (Shapiro and Wilk, 1965). A p-value >0.05 indicates normal distribution while a p-value <0.05 indicates abnormal distribution. In cases where there was significant evidence of non-normality, this could be ascribed to kurtosis rather than skewness. Interpretation of the results was thus continued (Glass, Peckham and Sanders, 1972). Treatment means were separated using Fishers' protected t-test least significant difference (LSD) at the 5 % level of significance (Snedecor and Cochran, 1980).

4.3 Results and Discussion

4.3.1 Feed

The vitamin A concentration in all five treatments was sampled weekly on day 7, day 14, day 21, day 28, day 35 and day 42 and analysed. Data was unbalanced. The independent variables were treatment, time and diet (TRM1, TRM2, TRM3, TRM4 and TRM5).

There was a significant treatment-diet effect (Table 4.3) for the starter to grower treatments. This is graphically depicted in Figure 4.2. The drop in retinol concentration in the starter diet to the concentration in the grower diet in TRM2 may have caused this effect. Therefore the effect of the different treatments on the starter (first three weeks) and the grower (last three weeks) had to be investigated separately.

Table 4.3: Comparison of the vitamin A concentration (mg/100g) between the different treatments for the starter and grower diets

	Starter (p-value = 0.2625)				
Level of treatment	**TRM1**	**TRM2**	**TRM3**	**TRM4**	**TRM5**
Mean	0.253	0.526	0.409	0.399	0.334
SD	0.056	0.306	0.259	0.314	0.209
n	6	12	7	11	8
	Grower (p-value = 0.0013)				
Level of treatment	**TRM1**	**TRM2**	**TRM3**	**TRM4**	**TRM5**
Mean	0.108^{bc}	0.018^{c}	0.156^{b}	0.285^{a}	0.086^{bc}
SD	0.051	0.010	0.078	0.189	0.077
n	6	6	7	10	8

(Note: Means with the same letter on a specific day are not significantly different)

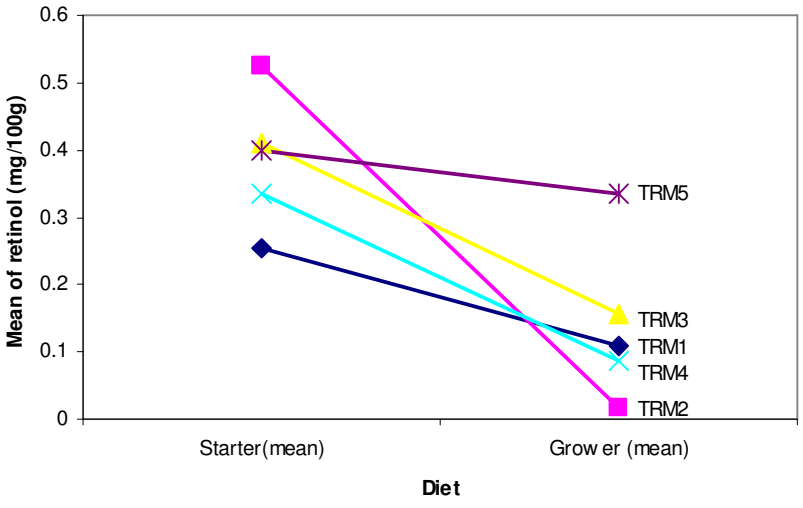

Figure 4.2: The treatment-diet effect from the starter diets to the grower diets

For the purpose of determining if vitamin A concentration decreased over time, the data of all treatments were pooled. A decrease in mean vitamin A concentration from day 7 to day 21 in the starter diet and from day 28 to day 42 in the grower diet was observed (Table 4.4). However, the decrease was not significant ($p>0.05$). Reasons for the variation in the vitamin A concentration within one treatment might be explained by inadequate mixing of the premix into the feed, segregation of the vitamin and the feed and storage losses (Blake, 2007). The variation in the fortified maize purchased from the retailers (TRM1 and TRM2) was discussed in the previous chapter. The quantitative difference in the vitamin A content of fortified white maize meal varied from the highest concentration of 226 µgRE/100g to the lowest concentration of <19 µgRE/100g.

Table 4.4: Comparison of the of vitamin A concentration (mg/100g) over time for the starter and grower diets

	Starter (p-value = 0.4872)			**Grower (p-value = 0.1653)**		
Level of time	**Day 7**	**Day 14**	**Day 21**	**Day 28**	**Day 35**	**Day 42**
Mean	0.469	0.399	0.349	0.180	0.154	0.091
SD	0.269	0.297	0.246	0.180	0.143	0.058
n	14	14	16	15	11	11

The theoretical vitamin A concentration in treatment 4 (TRM4) calculated from the formulation report of the premix supplier is 0.413 mg/100g (= 12 000 IU/kg) and 0.344 mg/100g (10 000 IU/kg) for the starter and grower diets respectively. Table 4.5 shows the analysed values per weekly interval for the different treatments. There was no significant difference at the 5% probability level in the vitamin A concentration within one treatment over time. This was expected as the feed for each treatment was mixed at the start of the feeding trial. There was also no significant difference ($p>0.05$) between the different diets within a certain week, which was not as expected. TRM1, TRM2 and TRM4 were formulated to have the same vitamin A content; while TRM3

was formulated to have a significantly higher (fortified and with premix) and TRM5 a lower (no fortification or premix) vitamin A concentration.

Table 4.5: Comparison of the vitamin A concentration (mg/100g) between the different treatments for the starter and grower diets over time

		Starter				Grower		
	[a]p-value	Day 7	Day 14	Day 21	[a]p-value	Day 28	Day 35	Day 42
TRM1								
Mean	0.6292	0.285	0.220	0.255	0.3379	0.155	0.090	0.080
SD		0.021	0.00	0.106		0.078	0.000	0.014
n		2	2	2		2	2	2
TRM2	0.3526				0.3720			
Mean		0.655	0.580	0.343		0.020	0.010	0.025
SD		0.345	0.334	0.205		0.014	0.000	0.007
n		4	4	4		2	2	2
TRM3	0.1325				0.2676			
Mean		0.470	0.203	0.655		0.127	0.235	0.12
SD		0.057	0.267	0.007		0.072	0.035	0.085
n		2	3	2		3	2	2
TRM4	0.1778				0.8373			
Mean		0.488	0.587	0.170		0.388	0.297	0.137
SD		0.300	0.314	0.242		0.222	0.175	0.050
n		4	3	4		4	3	3
TRM5	0.5002				0.2387			
Mean		0.245	0.230	0.430		0.105	0.065	0.070
SD		0.007	0.000	0.279		0.107	0.035	0.057
n		2	2	4		4	2	2
[b]p-value		**0.4134**	**0.2616**	**0.1964**		**0.0613**	**0.0937**	**0.2679**

[a]p-value for each treatment over time

[b]p-value for all the treatments within a week

n is the amount of analysis performed on a specific sample

Zeaxanthin and lutein are the major carotenoids in yellow maize, with β–carotene and β–cryptoxanthin being present in much smaller amounts (Rodriguez-Amaya and Kimura, 2004). The same pattern was found by Moros et al. (2002). Both lutein and zeaxanthin are not pro-vitamin A carotenoids and will therefore not have an effect on the overall vitamin A content of the yellow maize diets (TRM 4 and TRM5). In poultry nutrition these carotenoids are most often used for colouration of the egg yolk and skin (Castañeda, Hirschler, and Sams, 2005; Breithaupt, Weller and Grashorn, 2003). In

human health lutein and zeaxanthin are important in terms of their action against macular degeneration and cataract formation (Johnson, 2004).

Table 4.6 shows the cumulative feed intake for the different treatments over the six week period. There were no significant differences for the first seven days of the trial, but thereafter there were significant differences ($p \leq 0.05$) for cumulative feed intake. Treatment 4 had a significantly ($p \leq 0.05$) higher intake than the other four treatments whereas treatments 3 and 5 were significantly ($p \leq 0.05$) the lower. Treatment 4 had the highest cumulative feed intake followed by treatment 2.

Table 4.6: Cumulative Feed Intake for the chickens during a six week period on five different treatments

Level of Treatment	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
TRM1						
Mean	72.268	295.083^{b}	770.405^{a}	1317.203^{b}	1955.267^{b}	2899.128^{b}
SD	2.412	10.120	29.829	90.995	134.398	158.324
N	6	6	6	6	6	6
TRM2						
Mean	74.910	280.472^{b}	743.793^{a}	1461.745^{a}	2097.785^{ab}	3023.595^{ab}
SD	4.824	16.042	13.058	81.879	110.829	146.583
N	6	6	6	6	6	6
TRM3						
Mean	75.165	232.885^{c}	539.615^{b}	679.113^{c}	838.800^{c}	1140.260^{c}
SD	2.143	7.506	48.046	64.118	66.514	30.278
N	6	6	6	6	3	2
TRM4						
Mean	74.973	314.670^{a}	771.635^{a}	1495.317^{a}	2196.608^{a}	3237.460^{a}
SD	2.638	12.387	56.688	92.523	125.587	258.624
N	6	6	6	6	6	6
TRM5						
Mean	75.402	217.532^{d}	550.062^{b}	718.158^{c}	891.983^{c}	1095.273^{c}
SD	2.969	14.577	32.072	47.656	65.487	86.141
N	6	6	6	6	6	6
p-Value	**0.424**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**

(Note: Means with the same letter on a specific day are not significantly different)

n = values from six pens / treatments

Vitamin A concentration in TRM3 (with fortification and premix) was expected to reach possibly toxic levels and TRM5 (no fortification or premix) was expected to be a vitamin A deficient diet. If a diet is deficient in any nutrient, daily feed consumption may decrease in relation to the severity of the deficiency. If a diet has a gross excess of any nutrient, daily feed consumption usually also decreases in relation to the severity of the potential toxicity (NRC, 1994) as was observed in this study.

4.3.2 Body Weight

Table 4.7 and Figure 4.3 show the means of the body weights during the trial period. During the first 7 days there was no significant difference ($p>0.05$) in body weight of the chickens on the different treatments. This can be explained by the fact that the residual egg yolk provides nutrients to the chicks during the first few days after hatching. From day 14, treatment 4 (TRM4) produced significantly ($p\leq0.05$) higher bodyweights than the other four treatments. There were no significant differences ($p>0.05$) found between treatments 1 (TRM1) and 2 (TRM2) except at day 35. Treatments 3 (TRM3) and 5 (TRM5) were significantly ($p\leq0.05$) lower than the other treatments throughout the trial. There were no significant differences ($p>0.05$) between these two treatments (TRM3 and TRM5) except at day 14 and day 35. This correlates with the findings from Table 4.6. The cumulative feed intake was significantly lower and therefore the body weight is expected to be lower.

Table 4 7: Body weight of the chickens during a six week period on five different treatments

Level of Treatment	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
TRM1							
Mean	39.413	92.302^{b}	207.882^{b}	401.500^{b}	627.007^{b}	998.172^{a}	1153.035^{b}
SD	0.360	4.032	9.577	29.641	54.858	30.848	119.229
n	6	6	6	6	6	6	6
TRM2							
Mean	39.610	95.505^{ab}	209.050^{b}	415.270^{b}	632.110^{b}	929.058^{b}	1187.598^{b}
SD	0.499	4.298	8.175	12.219	6.827	48.834	44.144
n	6	6	6	6	6	6	6
TRM3							
Mean	39.580	98.245^{a}	186.330^{c}	270.550^{c}	313.160^{c}	297.223^{d}	469.000^{c}
SD	0.281	1.935	5.244	33.725	18.368	89.105	114.552
n	6	6	6	6	6	3	2
TRM4							
Mean	39.412	97.107^{a}	226.810^{a}	468.653^{a}	720.075^{a}	1034.760^{a}	1351.745^{a}
SD	0.217	3.210	6.513	11.359	9.149	28.912	83.602
n	6	6	6	6	6	6	6
TRM5							
Mean	39.567	96.512^{a}	170.535^{d}	259.368^{c}	326.813^{c}	365.515^{c}	485.972^{c}
SD	0.177	2.719	11.887	13.707	32.991	34.656	56.397
n	6	6	6	6	6	6	6
p-Value	**0.7248**	**0.0519**	**<0.001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**

(Note: Means with the same letter on a specific day are not significantly different)

n = values from six pens / treatments

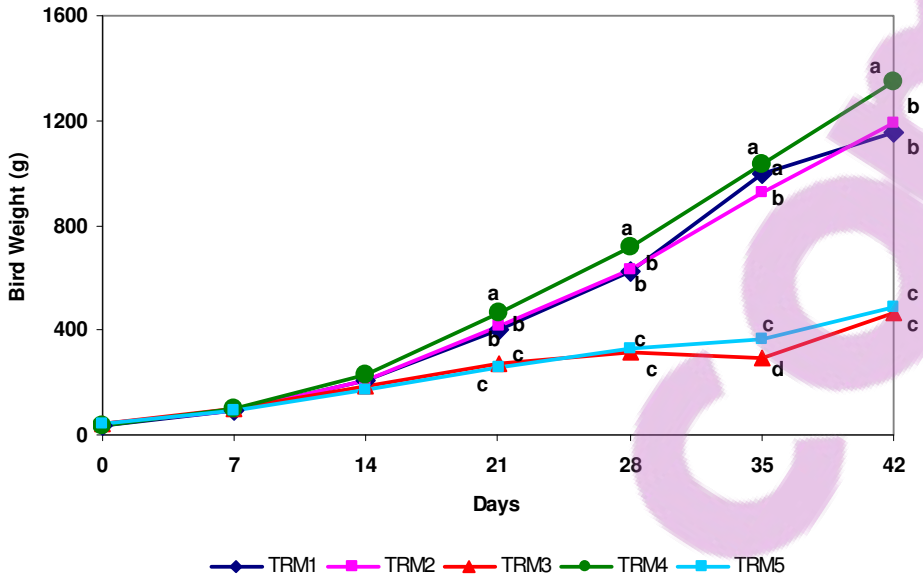

Figure 4.3: Means of body weight (grams) per week of broiler chickens on five different dietary treatments. (Note: Means with the same letter on a specific day are not significantly different)

4.3.3 Feed conversion ratio

Feed Conversion Ratio (FCR) for the different treatments are presented in Table 4.8. The feed conversion ratio (FCR) is a measure of an animal's efficiency in converting feed mass into increased body mass. Specifically FCR is the mass of the food eaten divided by the body mass gain, over a specified period of time. Poultry has a feed conversion ratio of 2 to 4 (FAO, 2006). The FCR for all the treatments is within this range from day 28.

There were no significant differences ($p>0.05$) during the first seven days. On day 14 treatment 3 had the lowest FCR ($p\leq0.05$). On day 35 treatment 1 (TRM1) had the lowest FCR, but there was no significant difference between treatment 1 (TRM1),

treatment 2 (TRM2) and treatment 4 (TRM4). Treatment 4 is an optimised poultry diet and the finding was as expected. Namely optimum weight gain with the lowest possible feed consumption (ie. low FCR). Therefore it can be assumed that the fortified white maize meal (TRM1 and TRM2) is as efficient in supplying the necessary nutrients to the chickens as the commercial poultry diet. During the last week of the trial the data shows no significant differences ($p>0.05$) among the treatments. However, the mortality (Table 4.9) was high for treatment 3 and 5 (TRM3 and TRM5). Therefore the results might not be a true reflection of body weight and FCR.

Table 4.8: Feed Conversion Ratio (FCR) for the chickens during a six week period on five different treatments

Level of Treatment	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
TRM1						
Mean	1.376	1.755^{a}	2.137^{b}	2.245^{b}	2.039^{c}	2.619
SD	0.149	0.083	0.156	0.083	0.124	0.212
n	6	6	6	6	6	6
TRM2						
Mean	1.348	1.657^{bc}	1.982^{b}	2.467^{a}	2.361^{c}	2.639
SD	0.149	0.093	0.077	0.134	0.103	0.193
n	6	6	6	6	6	6
TRM3						
Mean	1.283	1.588^{c}	2.359^{a}	2.481^{a}	3.434^{a}	2.743
SD	0.062	0.062	0.234	0.141	0.782	0.659
n	6	6	6	6	3	2
TRM4						
Mean	1.301	1.679^{ab}	1.799^{c}	2.197^{b}	2.206^{c}	2.469
SD	0.048	0.039	0.146	0.119	0.072	0.149
n	6	6	6	6	6	6
TRM5						
Mean	1.326	1.664^{bc}	2.505^{a}	2.521^{a}	2.763^{b}	2.476
SD	0.048	0.059	0.090	0.257	0.359	0.269
n	6	6	6	6	6	6
p-Value	0.5554	0.0081	<0.0001	0.0030	<0.0001	0.5007

(Note: Means with the same letter on a specific day are not significantly different)

n = values from six pens / treatments

4.3.4 Mortalilty

In table 4.9 the mortalities on day 21 and day 42 are shown. Mortalities for TRM3 and TRM5 are high and may be due to either a toxicity (TRM3) or a deficiency (TRM5) as previously discussed. In order to determine if this is true, cause of death should have been verified by separate analysis of the livers.

Table 4.9: Percentage mortalities during the trail period at day 21 and 42

Days	21	42
Treatments	%	%
1	1	1
2	1	2
3	22	71
4	2	2
5	12	64

4.3.5 Liver

The weekly liver samples, excluding mortalities, were weighed individually before freeze-drying. Figure 4.4 shows the liver weights during the trial period. There was no significant difference between the weights of the livers at baseline. After 14 days the mean liver weight from treatment 4 (TRM4) was significantly higher than treatment 3 (TRM3) and 5 (TRM5), but not significantly higher than treatments 1 (TRM1) and 2 (TRM2). This tendency was observed up to day 42.

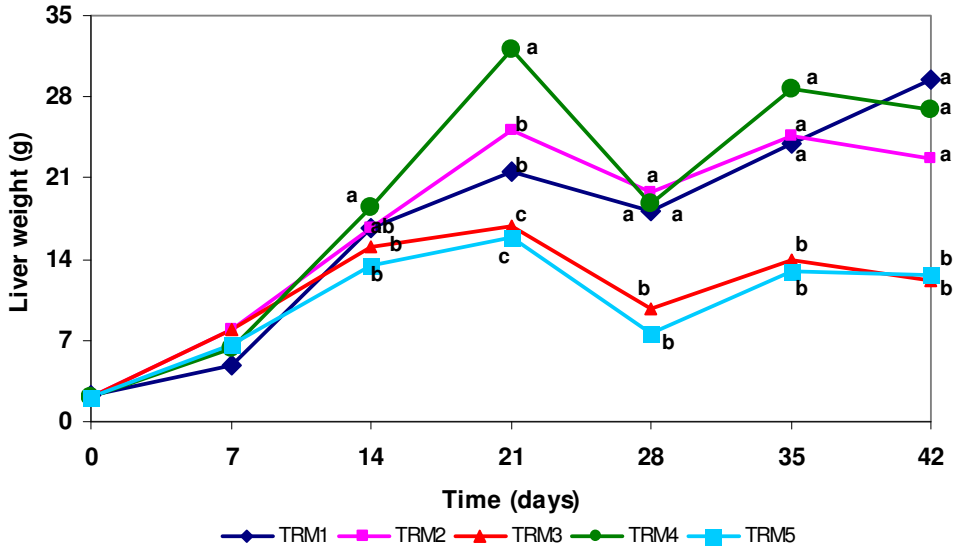

Figure 4.4: Comparison of means of liver weight (grams) per week of broiler chickens on five different dietary treatments. (Note: Means with different notations on a specific day are significantly different)

As expected with a fat-soluble vitamin, vitamin A levels in the liver must increase with time. However, during this study, the vitamin A levels in the livers of chickens on all the diets increased up to day 21 and decreased thereafter. It was also recognised that this was when the chickens changed from a starter to a grower diet. The decrease may be due to the diet. Although this decrease may also be due to a possible storage effect, as reported by Dos Santos et al. (2009) who found that vitamin A decreased in chicken livers stored for more than 30 days. Livers of a certain week in this study were analysed within a few days of each other. Therefore the effect of storage is for all treatments within a week and results can still be compared to study the absorption of vitamin A.

When comparing the liver vitamin A levels (Table 4.10) of the birds on the different treatments within a week, no significant difference ($p>0.05$) was observed at baseline. After the first phase of the trial (starter diets) TRM1 and TRM2 produced significantly

higher ($p \leq 0.05$) vitamin A levels in the livers, followed by birds on TRM4. The vitamin A concentration in the livers on day 21 of chickens on TRM1, TRM2 and TRM4 correlated with values found in livers of chickens on a diet containing 15 000 IU vitamin A/kg done by Lessard, Hutchings and Cave (1997). TRM3 and TRM5 chickens had the lowest vitamin A concentration in their livers. After 35 days there were no significant differences in vitamin A levels in the livers of birds on TRM1, TRM2 and TRM4 compared to TRM3 and TRM5 where the chickens had significantly lower vitamin A levels. As mortality (see Table 4.9) was high for TRM3 and TRM5 at 42 days the vitamin A content in the livers of the remaining birds are possibly not a true reflection of actual content due to the limited sample size.

Table 4.10: Average vitamin A (mg/100g) in the liver measured per week (comparing treatments within a week) of chickens on five different dietary treatments

		Starter			Grower		
	Day 0	**Day 7**	**Day 14**	**Day 21**	**Day 28**	**Day 35**	**Day 42**
TRM1							
Mean	0.685	6.340^{b}	9.146^{a}	14.088^{a}	5.599^{a}	4.504^{a}	2.958^{b}
SD	-	1.883	0.821	1.126	1.241	0.844	0.169
n	1	3	3	3	3	3	3
TRM2	0.590						
Mean	-	4.960^{bc}	8.912^{a}	12.049^{a}	5.314^{ab}	4.079^{a}	2.363^{bc}
SD	1	0.199	0.930	2.047	1.412	1.461	0.754
n		3	3	3	3	3	3
TRM3							
Mean	0.600	4.013^{c}	3.698^{c}	1.173^{c}	3.407^{b}	2.388^{ab}	5.196^{a}
SD	-	0.535	0.795	0.826	1.556	0.394	-
n	1	3	3	3	3	3	1
TRM4							
Mean	0.565	8.912^{a}	5.578^{b}	8.933^{b}	4.176^{ab}	4.196^{a}	4.768^{a}
SD	-	0.930	0.821	1.029	0.697	1.584	0.787
n	1	3	3	3	3	3	3
TRM5							
Mean		4.671^{bc}	2.253^{d}	0.331^{c}	0.583^{c}	1.601^{b}	1.250^{c}
SD	0.590	1.214	0.340	0.188	0.123	1.627	0.185
n	-	3	3	3	3	3	3
	1						
p-value		0.0022	<0.001	<0.001	0.0020	0.0713	0.003

Note: Means with different letters in a column are significantly different within a week

The area under the curve (AUC) was calculated using the vitamin A concentrations of the livers in Table 4.10. Due to high mortalities during the sixth week in TRM3 and TRM5, the AUC was only calculated up to day 35. Relative absorption was calculated using the diet optimised for the chickens (TRM4) as reference. Data is presented in Table 4.11 and graphically in Figure 4.5.

Tabel 4.11: Area under the curve (AUC) and relative absorption of vitamin A in chickens on five different diets over a six week period (p-value <0.0001)

	TRM1	TRM2	TRM3	TRM4	TRM5
Mean AUC	260.87[a]	232.26[a]	94.18[c]	178.89[b]	63.01[d]
SD	17.549	19.576	19.211	16.267	4.041
n	3	3	3	3	3
Relative absorption	1.46	1.3	0.53	1	0.35

Note: Means with different letters in a column are significantly different within a week

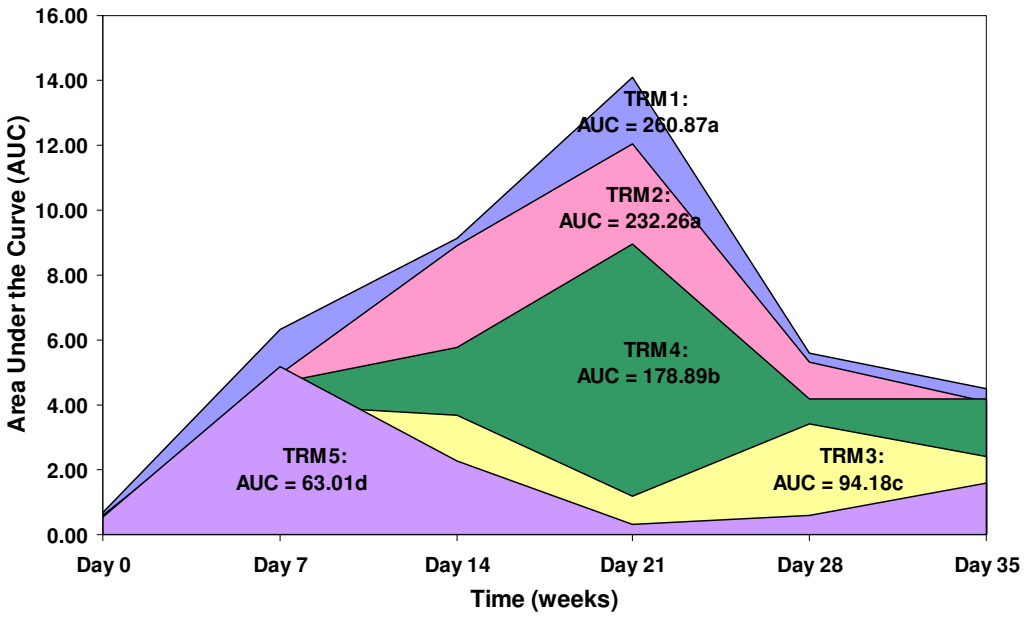

Figure 4.5: Means of AUC per week of broiler chickens on five different dietary treatments. (Note: Means with the same letter on a specific day are not significantly different)

The results show that the diet that was optimized for poultry nutrition (TRM4 – yellow maize with normal vitamin A supplementation) produced the highest weight gain and high cumulative feed intake. Chickens receive some endogenous nutrition (from the yolk) during the first week of life (NRC, 1994), therefore the treatment effect on body weight and liver weight only became evident after 14 days. Although the chickens on the diets with fortified white maize meal (TRM1 and TRM2) had a lower body weight than birds on TRM4, the body weight was still significantly higher than for TRM3 and TRM5. These two diets either had vitamin A added in addition to the fortificant in the fortified white maize meal (TRM3) or no vitamin supplementation to the yellow maize meal (TRM5). Birds on these two diets had the lowest feed intake resulting in lower body weights, lower liver weights and high mortality rates. This might suggest that the extra vitamin A in TRM3 could have deleterious effects in terms of possible vitamin A toxicity in chickens. However, this was not validated with analysis. Or it might be an issue of lower palatability of the diet as a result of the addition of the extra vitamin A. Chickens on TRM5 were vitamin A deficient with low vitamin A levels in the livers.

4.4 Conclusion

Although there was analytically no significant difference found in vitamin A levels in the different treatment diets, this study shows that a biological model is sensitive and can be used for evaluating dietary treatments. The suitability of a biological model for relative absorption/bioavailability was confirmed in this study.

Main findings observed are:

- The chickens performed optimally in growth and showed good vitamin A status in the liver without detrimental effects, when the supplementation was set at the optimal level;

- Results from the study show that vitamin A from fortified white maize can contribute as much vitamin A to the liver as a vitamin A supplement in the poultry diets;
- There is a significant difference in the vitamin A status of chickens consuming a low vitamin A diet vs. an adequate vitamin A diet;
- Optimal vitamin A intake is important to obtain a good vitamin A status.

Since there was no significant difference in vitamin A in the livers of birds on diets with the fortified white maize and the normal poultry diet, it can be assumed that the fortificant in the white maize is as absorbable as the vitamin A in the premix used in poultry nutrition. In translating these results to human nutrition, it is reasonable to conclude that the absorption of vitamin A in fortified maize meal is not a reason for the low vitamin A status of South African children five years after the implementation of mandatory fortification (NFCS-FB-I; 2008). Other reasons such as non-compliance by millers, the unavailability of fortified maize meal (e.g. farmers provide maize meal as part of remuneration to farm workers) or fortification levels set lower than the recommended dietary allowances (RDA) should be investigated.

It is important to note that this study was based on the consumption of raw maize meal by the chickens. An important difference between the diets of chickens and human diets, is the fact that the maize in a human diet is cooked prior to consumption changing the maize meal matrix. South African consumers mix maize meal with water, add a little bit of salt and heat the gruel until the starch is cooked. Although the water to maize porridge ratio might differ according to cultural preferences and the meal of the day, the preparation is similar. A thin watery porridge is usually eaten for breakfast and stiff porridge for the main meal of the day. Porridge is also cooked differently by either stirring a paste of maize meal mixed with cold water into the boiling water and covering

it until cooked; or by stirring it vigorously with a wisk for the full period, or variations thereof depending on culture.

4.5 Acknowledgements

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CHAPTER 5

SIGNIFICANCE OF THE STUDY, CONCLUSIONS AND RECOMMENDATIONS

This last chapter summarises the main findings of the research described. The implications of these findings and recommendations to consider in the future are presented and discussed.

5.1 Introduction

Micronutrient malnutrition contributes to a vicious cycle of poor health and depressed productivity, trapping families in poverty and eroding economic security in countries worldwide with vitamin A, iodine and iron deficiencies as amongst the world's most serious health risk factors. Vitamin A deficiency may also intensify a number of other health conditions, including anaemia (West, Gernand and Sommer, 2007). These broader health consequences further highlight the need to keep vitamin A deficiency controlled, especially during an economic crises. Ensuring adequate intake of this essential nutrient by vulnerable populations will offer enhanced protection from a range of disabilities and diseases, help children grow and learn, and improve health and productivity for adults.

As part of a food-based approach to alleviate micronutrient malnutrition the South African Directorate of Nutrition initiated a food fortification program (FFP). The Department of Health of South Africa embarked on a mandatory fortification program of the two staple foods, white maize meal and white and brown bread flour, with vitamin A, vitamin B_1 (thiamine), vitamin B_2 (riboflavin), vitamin B_3 (niacin), vitamin B_6

(pyridoxine), vitamin B_9 (folic acid), iron and zinc. The fortification level for vitamin A was established at 187.7 µgRE/100g maize meal to provide 31% of the RDA (Department of Health, 2003). The regulations imply that the final maize meal product shall comply with this level.

The aim of the research described in this thesis was to quantify the content of vitamin A in the fortified white maize meal as purchased from retail shelves, as well as that in the cooked porridge as traditionally prepared and consumed. The relative absorption of the vitamin A fortificant commercially used in white maize meal was also determined.

5.2 Significance of the study

5.2.1 Vitamin A content of fortified maize meal and porridge as purchased and consumed in South Africa

In the first study (Chapter 3) it was found that only one brand of white fortified maize meal from a sample of nine different brands complied with the regulatory requirement of 187.7 REµg/100g maize meal. This relates to a compliancy rate of only 11%. These results correlate well with the compliance rate of <20% recently reported by a study funded by the Global Alliance for Improved Nutrition (GAIN) (Umunna and Sunley, 2010). One brand of maize meal (11%) was fortified at a higher level than the mandatory level and seven brands (77%) reported lower values. The highest concentration found in this study was 261 µgRE/100g maize meal and the lowest mean vitamin A concentration was <19 µgRE/100g. The reasons for non-compliance may include:

- Millers do not fortify the maize meal according to standards. This may be a combination of many factors such as lack of competent personnel, lack of available funds to purchase the necessary equipment or analysis of vitamin A in final product on a regular basis;
- Poor uptake of the grant made available by the Department of Trade and Industry of South Africa for fortification equipment by small millers (de Hoop, 2010);
- Low quality fortification mixes supplied by unregistered fortification mix suppliers (DSM, 2009);
- Stability of vitamin A used in the fortification mixes (Mehansho *et al.*, 2003; WHO, 2009); and
- Storage conditions in supermarkets and small shops.

Vitamin A content was determined for each of the maize meal samples and the corresponding porridge samples. An average retention of 39.8% was found. Retention studies done to date conclude that a primary factor in vitamin A loss can be the moisture content in the premix and the maize meal, but that the different qualities of vitamin A compounds may also underlie wide variability in the vitamin A content (Klemm *et al.*, 2010).

To understand the contribution of the fortified maize meal to the vitamin A intake of children, the results were translated into Recommended Dietary Allowance (RDA) values. On average, approximately only 17% of the RDA for children 1-3 years and 13% of the RDA for children 4-9 years old were met by the fortification of the maize meal. This is on average 50% less than what the fortification program intended to contribute to the daily intake of children and it could be even lower depending on the portion size used in the calculation. In this study a portion size of 455 g/person/day for maize porridge was used (Steyn, Maunder and Labadarios, 2006). In a study reported

by Schönfeldt, Gibson and Vermeulen (2010) it was found that smaller portions (between 381g and 349g) are consumed, possibly as a coping strategy to counteract increased food prices.

5.2.3 Effect of different maize meal diets on the growth and vitamin A status of chickens

In the second study (Chapter 4) the use and suitability of a biological model were measured. Of importance when choosing an animal model is to always keep in mind the nutrient under investigation. In this study it was vitamin A. The following results were found:

- There is a significant difference in the vitamin A status of chickens consuming a low vitamin A diet vs. an adequate vitamin A diet;
- The chickens performed optimally in growth and showed good vitamin A status in the liver without detrimental effects, when the vitamin A level of the daily intake was set at the optimal level;
- Vitamin A in fortified white maize meal can maintain the vitamin A status of chickens;
- Optimal vitamin A intake is important to obtain a good vitamin A status.

The fortified maize meal diets were able to maintain the vitamin A status of the chickens. Poor absorbability or bioavailability of the fortified vitamin A is therefore not a constraint in combating vitamin A deficiency. It is therefore important to focus on the level of fortification delivered when the fortified food is consumed as a traditional prepared dish. In the traditional diet maize meal porridge is often consumed with a relish of dark green leafy vegetables, which contain phytates and oxalates which may have a negative influence of vitamin A absorption.

5.3 Concluding Remarks

In response to rising food prices, the poor (who are also most vulnerable to VAD) generally tend to purchase and consume smaller amounts of nutrient-dense foods, such as dairy, meat, eggs, fish, fruit and vegetables, while maintaining staple grain consumption despite the higher or fluctuating costs of the grain (Klotz, et al. 2008).

The results of this study indicate that fortification of commonly eaten staple foods with vitamin A could significantly improve the vitamin A intake of children under nine years of age and improve the overall micronutrient density of their diets. This is confirmed by the secondary data analysis of the national dietary data done by Steyn, Nel and Labadarios (2008). However, after five years of mandatory fortification, vitamin A deficiency (VAD) in South African children aged 1-9 years has worsened (NFSC-FB-I, 2008).

The 2005 National Food Consumption Survey- Fortification Baseline found that nearly two-thirds (64%) of children aged 1 – 9 years had a marginal or inadequate vitamin A status, and about one in seven children (14%) were severely vitamin A deficient. KwaZulu-Natal reported the highest proportion (89%) of children with an inadequate vitamin A status, with nearly half of the 1 – 9 year population severely deficient. Similarly, large proportions of children in the Limpopo (76%), Gauteng (65%) and Eastern Cape (64%) provinces had inadequate vitamin A status (NFSC-FB-I, 2008).

A marked increase in the prevalence of inadequate vitamin A status in children aged 1 – 5 years was observed. The national rate has nearly doubled between 1994 (33%) and 2005 (65%). Children aged 3 – 5 years are most affected. The National Food

Consumption Survey reports that, according to internationally accepted criteria, these high rates indicate that vitamin A deficiency is a serious public health problem in South Africa (NFSC, 2000; NFSC-FB-I, 2008).

Vitamin A status of the children was classified according to the World Health Organisation's criteria. Status was determined on the basis of the serum vitamin A concentration present in the blood drawn from children in the sample. Low serum vitamin A distributions (< 0.70 µmol/L) can be assumed to reflect chronic dietary inadequacy of vitamin A from preformed vitamin A and proactive carotenoid sources. However, status data do not provide information about the size of the dietary deficit, or gap, in requirements to meet via fortification or other dietary strategies.

One estimate of dietary gap is the added amount of vitamin A (in micrograms of retinol activity equivalents [µg RAE]) required to shift the intake distribution to the right of the estimated average requirement (EAR) so that only approximately 3% remain below that level in an age group. A second approach is to estimate the amount of vitamin A required to bring the mean of the population to the level of the recommended dietary allowance (RDA) (Klemm et al., 2010). The success of fortification of the second approach can be seen in the case of folic acid. The nutritional goal in the fortification program for folic acid was set at 50% of RDA as opposed to 31% of RDA for vitamin A. In the National Food Consumption Survey Fortification Baseline Study (NFSC-FB-I) done during 2005 an adequate folate status was reported throughout the country (NFSC-FB-I, 2008).

Either estimate of the dietary gap serves to represent the extent to which fortification should increase vitamin A intake to minimize the risk of deficiency. Both require quantified dietary intake data, preferably collected by repeated 24-hour recalls from a representative sample of a target population, assumptions of normality of usual intake

distributions, and an adequate food composition database. Unfortunately, few data of this nature, quality, and specificity exist (Klemm et al., 2010).

5.4 Limitations of the study

The following limitations of the study should be noted:

- Although 62 different fortified maize meal samples from nine different brands were analysed, the maize meal sample size (Chapter 3) was still small. To be more representative the number of samples and the brands covered could be increased. Maize meal from small, medium and large scale millers could be included. Such a study is currently being planned by the Department of Health and GAIN (Umunna and Sunley, 2010). For such a study to be successful, adequate funding, a comprehensive sampling plan together with the correct analytical techniques, are of utmost importance.
- Considering the results presented in Chapter 3 and although manuals on fortification of maize meal exist, it will be useful to include in the above-mentioned planned study a survey at the millers regarding *(i)* brand and product name of the fortification mix used, *(ii)* the general conditions under which the fortification mix is stored at the millers, *(iii)* how are the fortification mix added to the maize meal, *(iv)* how often are feeders calibrated, *(v)* the point during the milling process at which the fortification mix is added, and *(vi)* the type of quality control done on site.
- The use of animals as experimental models to gain insight into questions such as absorption of micronutrients (Chapter 4), is most valuable and informative. The ideal will still be to use human subjects to determine absorption and efficiency in a nutrition intervention trial.

5.5 Recommendations

An intervention to increase the micronutrient status of a population is an investment in human health and well-being. A multi-faceted approach is proposed throughout this thesis, including fortification, supplementation and dietary diversification as a sustainable approach to alleviate VAD in the world.

Fortification of foods with vitamin A is a potentially effective intervention to prevent or control vitamin A deficiency in low-income countries where undernutrition and poverty coexist.

The following points must be considered in the planning and execution of an efficient food fortification program:

- The fortification of a food with vitamin A should be designed to correct estimated dietary inadequacy in one or more vulnerable groups, that is, to fill a dietary gap (Klemm *et al.,* 2010). Actual portion size must be taken into consideration.
- The form of vitamin A and premix to be used in fortification should be the highest grade, appropriate for the intended food vehicle, stable under ambient conditions and for the duration of expected use, and introduced into the food supply in accordance with industry standards.
- Monitoring of compliance by all maize millers must be done on a regular basis by an independent organisation.
- Retailers must be trained on how to store and shelve the fortified product. The correct storage place or method can also be conveyed by means of a warning or instructions in different languages on the packaging. These instructions could then also inform the users at household level.

- Fortified products could be marketed through educational messages to children. Such messages should explain the health benefits when these products are consumed. The fortification logo should be used as a marketing tool to create consumer-demand for the fortified product.

The total diet should always be considered when deciding on fortification levels. According to the NFSC (2000), the five most often consumed foods are maize porridge, brown bread, black tea, sugar and a small amount of full cream milk. It is recommended that nutrient content and bioavailability of maize porridge as consumed traditionally with a relish of dark green leafy vegetables be determined. The fibres, oxalates and phytates in the dark green leafy vegetables may interfere with absorption. If tea is the beverage consumed with the meal, the tannins in the tea can also interfere with micronutrient absorption. From a nutritional point of view there are not many absorption enhancers in the abovementioned diet apart from the small amount of milk consumed.

The availability of micronutrient-dense maize might be a suitable alternative to fortified maize meal. Biofortification of cereal staples could have broad potential for ensuring dietary vitamin A adequacy in vulnerable populations by increasing β-carotene intake from readily absorbable staple grain matrices (Graham and Rosser, 2000). Gradually those being reached by biofortified crops might reduce the need for commercial fortification. Biofortification can reach rural populations effectively, and commercial fortification can reach urban populations effectively. The acceptability of such approaches by the identified populations at risk of Vitamin A deficiency, needs further investigation.

Establishing a sustainable vitamin A adequate diet by selecting appropriate food throughout an individual's life cycle remains a challenge particularly amongst the poor

in developing countries. These populations often escape the safety nets of government supplementation and fortification programs. It is strongly recommended that international agencies such as the FAO, WHO, UNICEF and GAIN continue to encourage governments to assist individuals at risk of developing VAD with suitable alternatives. This is not only a part of every person's human right to adequate nutritious food, but should also decrease the burden of disease. This is in line with Goal 1 of the millennium development goals: Eradication of extreme poverty and hunger, (MDG's) (UN, n.d.).

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ADDENDUM A

Nutrient content of South African white maize meal (unfortified) (Wolmarans, Danster and Chetty, 2005)

	Macronutrients					Minerals			Vitamins			
	Moist-g AnPr-g Chol-mg	En-kJ Fat-g CHO-g	TotN-g SFA-g TFib-g	Prot-g MFA-g AdSu-g	PlPr-g PFA-g Ash-g	Ca-mg P-mg Zn-mg	Fe-mg K-mg Cu-mg	Mg-mg Na-mg Mn-µg	A-µgRE B6-mg Biot-µ	Thia-mg Fol-µg C-mg	Ribo-mg B12-µg D-µg	Niac-mg Pant-mg E-mg
Maize meal, sifted, raw (white, unfortified)	11.9 0.0 0	1355 3.3 68.0	1.33 0.46 7.3	8.3 0.90 0.0	8.3 1.43 0.9	3 190 1.67	1.5 196 0.16	98 3 360	12 0.31 7.4	0.38 29 tr	0.05 0.0 0.00	1.7 0.44 0.56
Maize meal, special, raw (white, unfortified)	11.6 0.0 0	1380 2.9 71.5	1.23 0.38 5.5	7.6 0.81 0.0	7.6 1.13 0.8	6 105 1.53	1.2 180 0.22	83 7 540	23 0.37 3.1	0.38 42 tr	0.05 0.0 0.00	1.6 0.39 0.49
Maize meal, super, raw (white, unfortified)	12.0 0.0 0	1360 1.4 74.0	1.23 0.19 4.9	7.7 0.28 0.0	7.7 0.42 0.5	3 60 0.58	0.7 108 0.10	36 3 170	tr 0.17 3.8	0.13 8 tr	0.11 0.0 0.00	0.8 0.31 0.34

ADDENDUM B

A list of brands of maize meal samples analysed in the study

Code	Brand Name	Type of maize meal
A	Ace	Super
B	Impala Special	Special
C	Iwisa	Super
D	Pride	Super
E	Super Sun	Super
F	Tafelberg	Super
G	White Star	Super
I	5 Star	Special
J	A1	Super

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ADDENDUM C

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Date: 16th April 2010

Dear Ms B. Pretorius

Re: Inquiry on Animal Ethics ref no for project "A comparison of the efficacy of fortified white maize meal and yellow maize meal in improving the vitamin A status of vitamin A deficient chickens".

Your project entitled "A comparison of the efficacy of fortified white maize meal and yellow maize meal in improving the vitamin A status of vitamin A deficient chickens" was submitted for evaluation in 2007 and approved. Its reference number is **APIEC07/01**

Regards

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