EFFECT OF DIFFERENT MAIZE MEAL DIETS ON GROWTH AND VITAMIN A : CASE-STUDY ON CHICKENS

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Abstract

BACKGROUND: South Africa embarked on mandatory vitamin and mineral fortification of wheat flour and maize meal in 2003 as part of a multi-faceted approach to alleviate malnutrition. However it was reported in 2008 that vitamin A deficiency increased despite the mandatory fortification programme. This motivates the investigation into the absorption of the vitamin A as fortificant in the maize meal.

Relative absorption in chickens as the biological model was determined by evaluating growth and vitamin A status. The weight, cumulative feed intake and liver retinol stores of the chickens on different diets were measured over a six week period.

RESULTS: The fortified white maize meal diet was able to maintain the vitamin A status of the chickens.

CONCLUSION: Poor absorption of the fortificant vitamin A is therefore not a constraint in combating vitamin A deficiency. It is in therefore also important to focus on the level of fortification delivered when consumed as a traditional prepared dish. In the traditional diet maize porridge is often consumed with only a relish. The total fat content of the traditional meal is very low, lacking absorption enhancers.

Keywords: Fortification, Vitamin A, Maize Meal, Porridge, Absorption

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 y consume <50% of the recommended dietary intake of vitamin A (700 µg retinol equivalents) (NFCS, 2000). Children living in rural areas being 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 animal feed. The National Food Consumption Survey (NFSC, 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. 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 B_6 and riboflavin since October 2003 as part of a multi-faceted approach to alleviate malnutrition. The final, minimum level of vitamin A in fortified maize meal at 12.5% moisture basis shall be not less than 1877 µgRE/kg (Department of Health, 2003). One of the

considerations in a fortification program is the 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, Boileau, Boileau, Williams, Swanson, Heintz and Erdman, 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, 1999).

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, cumulative feed intake and liver retinol stores of the chickens on different diets over a six week period.

Materials and Methods

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; protocol approval number ApIEC07/01. 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°C). The vaccination programme applied was according to the Poultry Reference Laboratory at the University of Pretoria, Onderstepoort (2002). 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 (Table 1). 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 (137 Terrace Road, Sebenza, 1610, South Africa) a company supplying vitamin and mineral premixes for animal nutrition.

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 vitamin A concentrations in the liver at the start of the study. 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 ARC-Irene Analytical Services, Irene, South Africa for determination of the vitamin A concentration.

Measurements and observations:
Birds: Birds were weighed weekly on a per pen basis starting from day 0 until 42 days of age.
Feed: Feed samples per treatment were taken weekly and stored in a freezer at 4°C until vitamin A was determined. All analyses were done in duplicate. **Actual average feed intake per bird was determined by weighing back feed on a weekly basis.**

Table 1: Diet formulation (%) for standardised starter and grower diets, showing source of maize meal and source of vitamin A per treatment

Starter					Treatments	Grower/Finisher				
TRM1	TRM2	TRM3	TRM4	TRM5		TRM1	TRM2	TRM3	TRM4	TRM5
60.84					**Treatment 1 (TRM1)** Fortified white maize meal (Brand F) with normal vitamin and mineral premix optimised for chickens; without vitamin A supplementation	72.92				
	60.84				**Treatment 2 (TRM2)** Fortified white maize meal (Brand A) with normal vitamin and mineral premix optimised for chickens; without vitamin A supplementation		72.92			
		60.84			**Treatment 3 (TRM3)** Fortified white maize meal (Brand B) with normal vitamin and mineral premix optimised for chickens; with vitamin A supplementation			72.92		
			60.84		**Treatment 4 (TRM4)** Yellow maize meal with normal vitamin and mineral premix optimised for chickens; with vitamin A supplementation				72.92	
				60.84	**Treatment 5 (TRM5)** Yellow maize meal with normal vitamin and mineral premix optimised for chickens; without vitamin A supplementation					72.92
3.96	3.96	3.96	3.96	3.96	Sunflower OK	*	*	*	*	*
19.86	19.86	19.86	19.86	19.86	Soyabean OK	12.82	12.82	12.82	12.82	12.82
11.37	11.37	11.37	11.37	11.37	Maize Gluten 60	10.39	10.39	10.39	10.39	10.39
2.16	2.16	2.16	2.16	2.16	Limestone	2.24	2.24	2.24	2.24	2.24
0.39	0.39	0.39	0.39	0.39	Salt	0.25	0.25	0.25	0.25	0.25
0.14	0.14	0.14	0.14	0.14	L Lysine HCL	0.10	0.10	0.10	0.10	0.10
0.20	0.20	0.20	0.20	0.20	DL Methionine	0.20	0.20	0.20	0.20	0.20
0.50	0.50	0.50	0.50	0.50	Mono Ca P	0.50	0.50	0.50	0.50	0.50
		0.50	0.50		Vitamin & Minerals (With vitamin A)			0.50	0.50	
0.50	0.50			0.50	Vitamin & Minerals (Without vitamin A)	0.50	0.50			0.50
0.05	0.05	0.05	0.05	0.05	Salinomycin	0.05	0.05	0.05	0.05	0.05

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

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.

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). The concentration of vitamin A was determined as described by Hulshof (2002). The basic method entails the alkaline saponification of the test material to eliminate the fat and to liberate the natural retinol in the cells. This is followed by ether extraction of the retinol and determination by high performance liquid chromatography (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. Separation was achieved using a mobile phase of 97% methanol in deionised water and a flow rate of 1.0 mL/min. Separations was performed at 325 nm for the identification and quantification of retinol.

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. A p-value <0.05 indicates abnormal distribution. In cases where there was still 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).

Results and Discussion

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).

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. 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 and a small sub-sample that was taken for analysis (Blake, 2007).

Table 2: Comparison of the vitamin A concentration (mg/100g) in starter and grower diets for the different treatments over time

	Starter				Grower			
	Day 7	Day 14	Day 21	[a]p-value	Day 28	Day 35	Day 42	[a]p-value
TRM1								
Mean	0.285	0.220	0.255	0.6292	0.155	0.090	0.080	0.3379
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

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 2 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.

No carotenoid analysis was performed on the maize meal or the diets. 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, Darnoko, Cheryan, Perkins and Jerrell (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 is most often used for colouration of the egg yolk and skin. In human health lutein and zeaxanthin is important in terms of their action against macular degeneration and cataract formation (Johnson, 2004).

Table 3 shows the cumulative feed intake for the different treatments over the 6 week period. There were no significant differences for the first seven days of the trial, but thereafter there were significant differences (p≤0.05) for cumulative feed intake. Treatment 4 had a significantly (p≤0.05) higher intake than the other four treatments whereas treatments 3 and 5 were significantly (p≤0.05) the lowest. Treatment 4 had the highest cumulative feed intake followed by treatment 2.

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, 1999) as was observed in this study.

Table 3: 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	72.3	295.1^b	770.4^a	1317.2^b	1955.3^b	2899.1^b
SD	2.41	10.1	29.8	91.0	134	158
n	6	6	6	6	6	6
TRM2						
Mean	74.9	280.5^b	743.8^a	1461.7^a	2097.8^{ab}	3023.6^{ab}
SD	4.82	16.0	13.1	81.9	111	147
n	6	6	6	6	6	6
TRM3						
Mean	75.2	232.9^c	539.6^b	679.1^c	838.8^c	1140.3^c
SD	2.14	7.51	48.0	64.1	66.5	30.3
n	6	6	6	6	3	2
TRM4						
Mean	75.0	314.7^a	771.6^a	1495.3^a	2196.6^a	3237.5^a
SD	2.64	12.4	56.7	92.5	125	258
n	6	6	6	6	6	6
TRM5						
Mean	75.4	217.5^d	550.1^b	718.2^c	892.0^c	1095.3^c
SD	2.97	14.6	32.1	47.7	65.5	86.1
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)

Body Weight

Figure 1 shows the mean of the body weight of the chickens 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 chick during the first few days after hatching. From day 14, treatment 4 (TRM4) produced significantly (p≤0.05) higher body weights than the other four treatments. There were no significant differences (p>0.05) found in bodyweights between treatments 1 (TRM1) and 2 (TRM2) except at day 35. Chickens on treatments 3 (TRM3) and 5 (TRM5) have significantly (p≤0.05) lower bodyweights than the chickens on 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 3. The cumulative feed intake was significantly lower and therefore the body weight is expected to be lower.
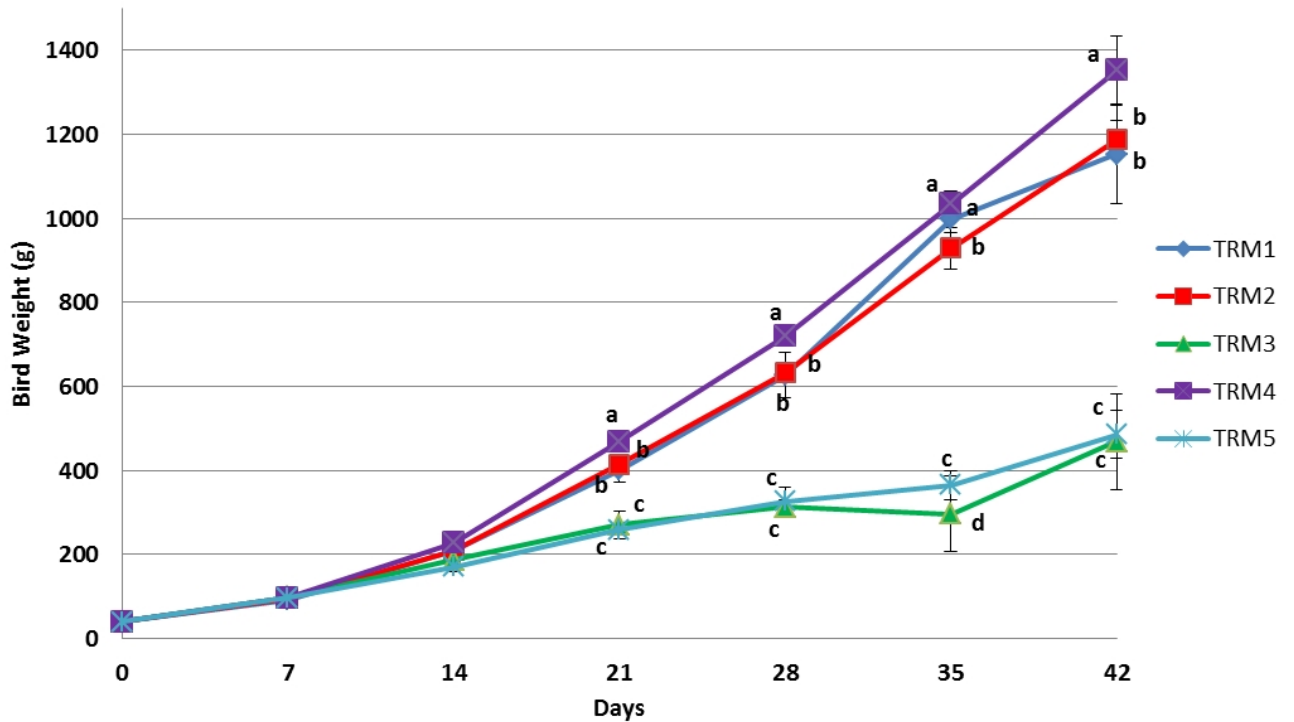
Figure 1: Means of body weight (grams) per week of broiler chickens on five different dietary treatments. (Note: Means with the same letter on a specific day are not significantly different)

Liver

All the liver samples were weighed individually before freeze-drying. 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. Results are not shown.

The average vitamin A concentration in the livers correlates with the normal vitamin A concentration (5.60 ± 3.89 mgRE/100g) in the livers of chickens in a study done by Majchrzak, et al (2006). A reason for the observed variation of vitamin A stored in chicken livers is the amount of the vitamin A contained in the feed. The vitamin A levels in the livers of chickens on all the diets increased up to day 21 and decreased when the chickens changed from a starter to a grower diet. This decrease may be due to the diet or due to a possible storage effect, as reported by Dos Santos, Da Costa, Soares, Pires, Ramalho and Dimenstein (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.

Table 4: 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

When comparing the liver vitamin A levels (Table 4) 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≤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 chicken livers of chickens on a diet containing 15 000 IU vitamin /kg done by (Lessard, Hutchings and Cave, 1997). Chickens on TRM3 and TRM5 had the lowest vitamin A concentration in their livers. After 35 days there was no significant difference 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 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.

Conclusion

Although the chickens on the diets with fortified white maize meal (TRM1 and TRM2) had a lower body weight than birds on TRM4, there was no significant difference in vitamin A concentration in the livers of the chickens on these three diets. Therefore 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. It is therefore important to focus on the level of fortification delivered when the fortified food is consumed as a traditional prepared dish. Actual portion size and absorption enhancers and inhibitors must be taken into consideration. In the traditional diet maize meal porridge is often consumed with only a relish of dark green leafy vegetables, high in dietary fibre, phytates and oxalates which may have a negative influence on mineral absorption, such as iron, calcium and magnesium. 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 traditional diet apart from a small amount of milk consumed. However more important to consider is the total fat content of the meal which is traditionally very low. According to the National Food Consumption Survey (NFSC, 2000), for South Africa as a whole the mean intakes of the fat soluble vitamins measured, vitamin A, D and E was very low compared to the Daily Recommended Intake (DRI's).

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