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)

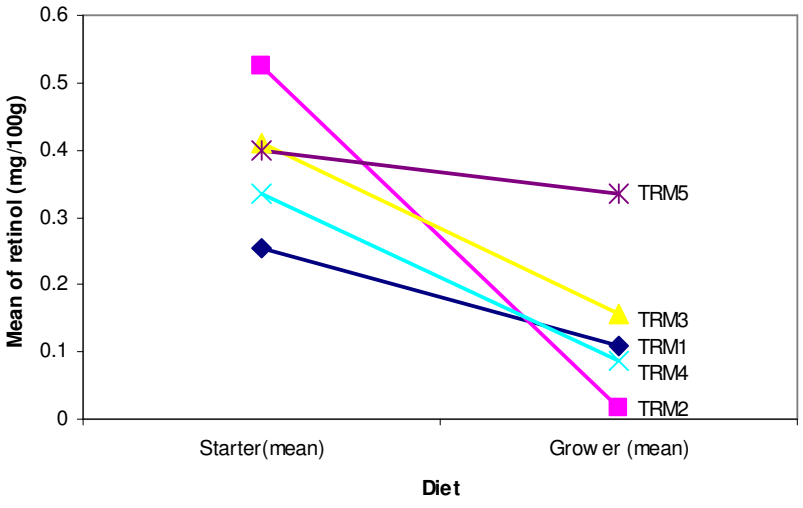

Figure 4.2: The treatment-diet effect from the starter diets to the grower diets

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

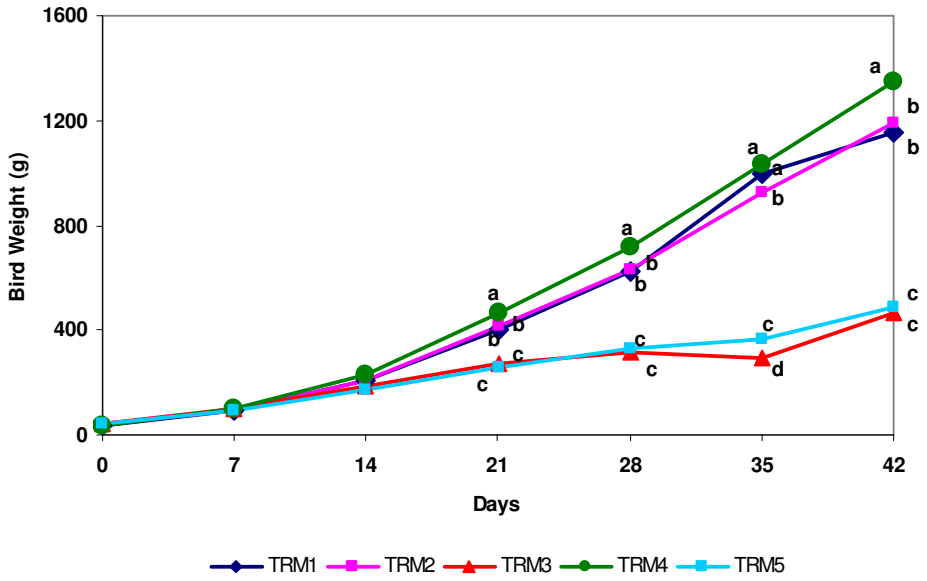

Figure 4.3: Means of body weight (grams) per week of broiler chickens on five different dietary treatments. (Note: Means with the same letter on a specific day are not significantly different)

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.

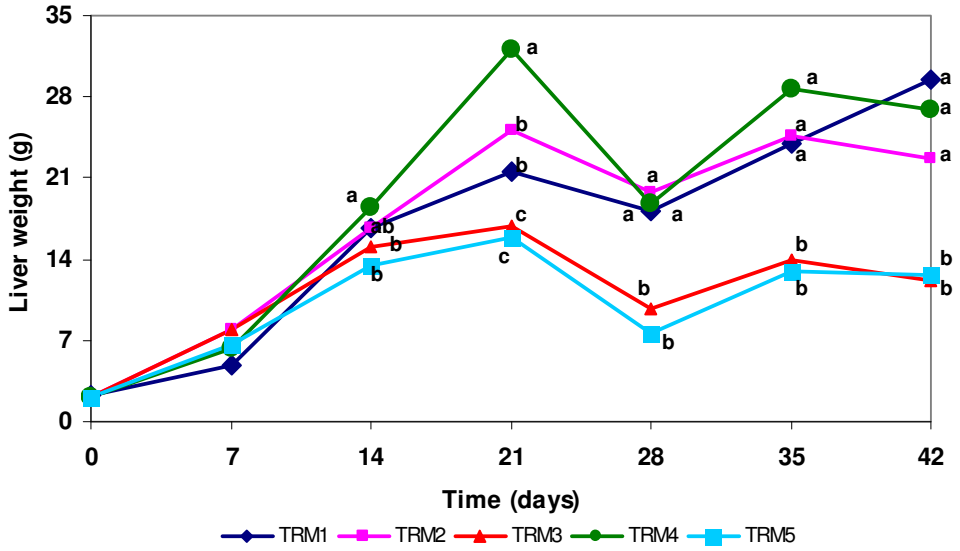

Figure 4.4: Comparison of means of liver weight (grams) per week of broiler chickens on five different dietary treatments. (Note: Means with different notations on a specific day are significantly different)

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

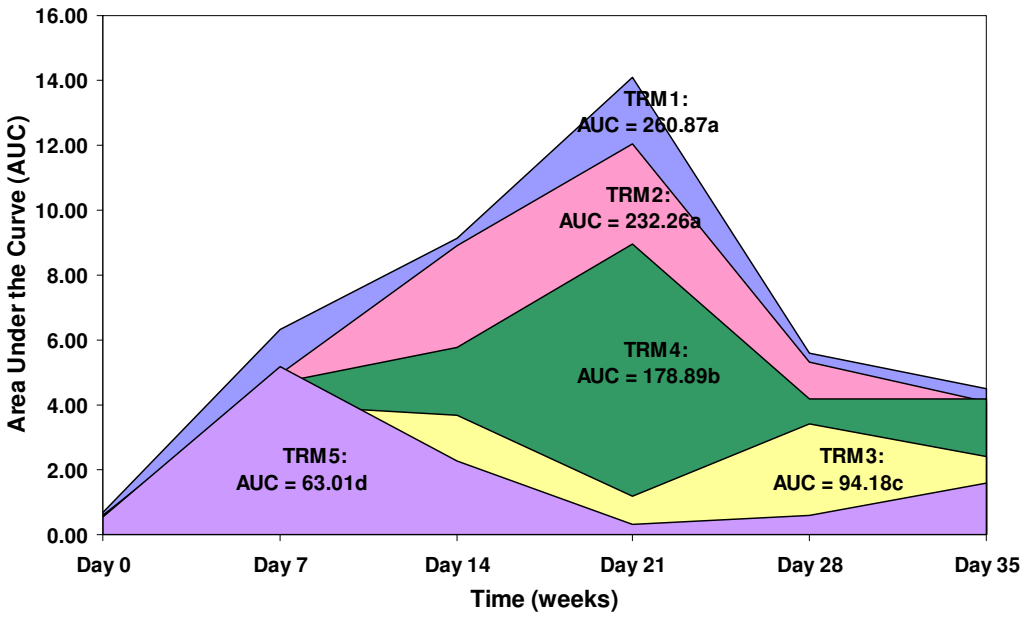

Figure 4.5: Means of AUC per week of broiler chickens on five different dietary treatments. (Note: Means with the same letter on a specific day are not significantly different)

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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