NUTRITIVE EVALUATION OF GRAIN AMARANTH (Amaranthus spp.) IN BROILER CHICKEN DIETS

BY

LUCY WANGECHI KABUAGE

A THESIS SUBMITTED IN FULFILLMENT FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN ANIMAL PRODUCTION IN THE FACULTY OF AGRICULTURE, UNIVERSITY OF NAIROBI.

1996

DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

Signed: _____ Date: 8th Oct. 1996
LUCY W. KABUAGE

This thesis has been submitted for examination with our approval as University supervisors.

Signed: _____ Date: 8/10/96
PROF. P.N. MBUGUA
DEPT. OF ANIMAL PRODUCTION

Signed: _____ Date: 8/10/96
PROF. B.N. MITARU
DEPT. OF ANIMAL PRODUCTION;
INSTITUTE OF DRYLAND RESEARCH,
DEVELOPMENT AND UTILIZATION

Signed: _____ Date: 14/10/96
DR. T.A. NGATIA
DEPT.OF VETERINARY PATHOLOGY AND
MICROBIOLOGY

ABSTRACT

Studies were carried out to determine the nutritive value of grain amaranth as a feed ingredient for broiler chickens and assess the extent to which it could replace maize in broiler diets. Three grain species were evaluated namely, *Amaranthus cruentus* L., *Amaranthus hypochondriacus* L. and *Amaranthus caudatus* L.. In the preliminary study, the chemical attributes of the grain were assessed. The average crude protein and metabolisable energy contents of the three grain species were 15.80% and 3310 kcal/kg, respectively. The mean lysine and sulphur amino acid levels were 0.78 and 0.67%, respectively. The mean tannin and trypsin inhibitor contents were 0.093% and 0.65 trypsin inhibitor units/mg, respectively. *A. caudatus* species had superior nutrient composition but contained the highest levels of antinutritional factors.

In Experiment 1, raw grain from each of the three species was incorporated in broiler starter diets at 20, 40 and 60% levels. Chick body weight and feed intake at four weeks of age declined ($P<0.05$) while pancreas weights increased with increasing levels of dietary amaranth. The 20% *A. hypochondriacus* diet however gave similar ($P>0.05$) chick performance to that of the maize-soyabean meal control diet. Upon thermal extrusion of grain amaranth in Experiment 2, chick performance markedly improved, with the 20 and 40% *A. hypochondriacus* and *A. cruentus* grain diets showing similar ($P>0.05$) body weight, feed intake and feed efficiency as the maize-soya bean meal control diet.

In Experiment 3, inclusion of lysine, lysine plus methionine and casein in 40% raw and 60% thermal processed *A. hypochondriacus* diets showed similar ($P>0.05$) chick performance between the thermal processed amaranth diet containing casein and

the maize-soya bean meal control diet. The latter diet however, resulted in higher ($P<0.05$) essential amino acid availability. Casein increased the levels of various essential amino acids in the amaranth diets. Chicks on thermal processed amaranth diets were heavier and consumed more feed than those on diets containing raw grain amaranth. The low zinc retention from all the diets indicated interference with absorption of this mineral. In Experiment 4, casein and ethylene diamine tetraacetate (EDTA) were separately added to 40% raw and 40% thermal processed *A. hypochondriacus* diets. Chicks on the processed amaranth diets gave higher ($P<0.05$) body weight, feed intake and nitrogen retention. Inclusion of EDTA in amaranth diets failed to increase ($P>0.05$) mineral retention but the tissue mineral content of chicks was adequate.

In Experiment 5, 20 and 40% *A. hypochondriacus* diets with or without molasses were fed either as raw grain mash diets or in steam pelleted form. Chick body weight was higher ($P<0.05$) for the pelleted and maize control diets at 4 weeks of age. However, continued feeding up to 8 weeks of age resulted in similar ($P>0.05$) chick body weights for all the diets including the maize control. Pelleting improved ($P<0.05$) body weight and feed efficiency and increased ($P<0.05$) carcass fat but molasses inclusion had no effect ($P>0.05$) on feed intake or body weight. Histopathological studies of chick organs did not show changes attributable to amaranth feeding.

Growth depression in chicks fed on raw amaranth diets mainly resulted from reduced feed intake accompanied by low protein digestibility. Raw and thermal processed *A. hypochondriacus* grain can effectively replace maize up to 20 and 40% of broiler starter diets, respectively.

ACKNOWLEDGEMENTS

I wish to express my deep gratitude to Prof. P.N. Mbugua for the invaluable assistance and guidance he kindly offered throughout the research study and writing of the thesis. I am also sincerely grateful to Prof. B.N. Mitaru and Dr. T.A. Ngatia for their assistance, advice and support. I highly appreciate the tireless efforts of the three supervisors in ensuring that the entire study was thoroughly accomplished.

I would like to thank the Chairman of the Department of Animal Production where the study was carried out and all the staff in the department who assisted in one way or another. Special thanks go to the staff of the poultry unit and the laboratory, especially to Mrs M.Wangai, Mr.S.Wachira, the late B.Chebe and Messrs J.Shivachi, M.Angala, D.Ambale, N.Kahiga and J.M.Kanyi.

I am grateful to Mr. Matere in the Department of Public Health, Pharmacology and Toxicology (PHPT) for his valuable input in statistical data analysis, Mr. John Gitau and Miss Beatrice Ndung'u for wordprocessing and Mr. T.Njau for assisting with routine project duties. Many thanks for the technical assistance received from the Department of Veterinary Pathology and Microbiology and Department of Food Technology and Nutrition pilot plant. I acknowledge the help from Messrs J.Waweru, Wamakima, Muthami and Ngige in the two departments.

I express my gratitude to Unga Feeds Ltd. for supplying most of the feed ingredients and their kind provision of pelleting facilities at minimum cost. Special thanks to Mr. Farthing, Mr. J.P. Magadi and the Nakuru branch manager, Mr. Kariuki. I wish

to thank Mr. David Mwangi of Nanyuki Natural Foods Company for providing the grain amaranth and Supa Snacks Ltd. for extrusion facilities.

I would like to thank the Ministry of Agriculture, Livestock Development and Marketing especially the Deputy Director (Training and Extension) Dr. L.W.Kimaru, the former Principal of AHITI-Kabete Dr. J.K.Gachaki and the current Principal Dr. J.M.Kamau, for their role in facilitating the granting of my study leave.

My very sincere thanks go to the German Academic Exchange Service (DAAD) for awarding me a scholarship for the PhD study including a study visit to the Institute of Tierernahrung in Freie University, Berlin, Germany where work on amino acid analysis was carried out. Very special thanks to the Director of the Institute Prof. Schneider for use of facilities, Dr. K. Schafer for organising and supervising my programme with keen interest, Mrs A. Lenke for daily assistance in the laboratory and Mrs. K. Meyer for accommodation and generous hospitality throughout my stay in Berlin.

My family deserves special mention for their never ending love, patience, understanding and great support throughout the period of study. My deepest appreciation to my husband Sam and children, Maina, Mutero, Wangari and Njoroge, who constantly encouraged me in my endeavour to accomplish this noble goal.

Finally, I wish to thank all those nice people who assisted or encouraged me in any way, especially my friends and colleagues, but most of all my relatives whose genuine concern kept me hopeful and optimistic.

DEDICATION

To my beloved parents Mr and Mrs F. Mutero who inspired every child in the family to strive for the highest goals in the education system.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

APPENDIX

Grain amaranth crop at the field station, Kabete campus, University of Nairobi.

CHAPTER 1

1.0 INTRODUCTION

The poultry industry in Kenya is changing due to the market liberalisation policies currently being implemented. Trade restrictions previously imposed on importation of day old chicks, feed ingredients and other resources needed in the poultry industry have been relaxed. The extent to which the industry may expand in future will hence be regulated by the existing market forces.

Poultry consume about 70% of the total animal feed manufactured locally. The availability of high quality feed is linked to an adequate supply of appropriate raw materials. The main energy ingredients used are maize, wheat and their by products. Oil seed cakes, fish and meat meals are the major sources of protein. However, Kenya has not been self sufficient in these feedstuffs. Maize forms the staple food for a majority of the local population. Its use in manufacture of animal feeds is thus of secondary importance. Production of oil seed crops has declined due to a poor marketing environment that has led to lack of farmer incentives. The deficit in supply of these feed resources is bridged through importation.

There is need to search for alternative energy and protein ingredients locally to meet the increasing demand from both human and livestock populations. One crop that could be

considered suitable for this purpose is of the genus amaranthus which yields a highly nutritious grain. Grain amaranth (Amaranthus spp.) has the potential to be adapted for food use under Kenyan agricultural conditions (Gupta and Thimba, 1992). There has been emphasis on production of drought resistant crops like sorghum, millets and cassava which can grow in the arid and semi arid areas of Kenya. When used as feedstuffs these crops mainly supply energy and require supplementation with protein rich ingredients to make well balanced feed. Grain amaranth has the potential to substitute or complement cereals due to its high energy and protein content and good amino acid composition. Its low water requirement for growth makes it suitable for the marginal areas of Kenya. Although some agronomical data on grain amaranth grown locally is available, there is hardly any information on its use as a poultry feed.

Grain amaranth was first grown by the Incas and Aztecs in Central America during pre-Columbian times. The crop's natural hardiness and high nutritional value of its grain and greens have recently attracted an increasing amount of interest from agronomists, nutritionists and health food industries in the United States of America. A 1975 National Academy of Science study identified grain amaranth as an under exploited plant with economic potential and concluded that multidisciplinary research efforts should be initiated with the aim of exploiting the crop (NAS, 1975). Work done to establish the nutritive value of the grain showed that it

possibly contained certain antinutritional factors that caused poor animal performance (Cheeke and Bronson,1980; Pond *et al.* 1991). The adverse effects were partly overcome when the grain was heat processed (Cheeke and Bronson, 1980; Tillman and Waldroup, 1986). More research is required to evaluate grain amaranth as an ingredient for poultry feeds and determine whether it can be an effective substitute for maize grain. Different methods of processing that can enhance its nutritive value need to be established. Information on optimal levels of amaranth inclusion could enlighten the feed manufacturer on appropriate ways of utilising this grain in compounding feeds for poultry.

1.1 Objectives

The overall objective of this study was to determine the efficacy of grain amaranth as a feed ingredient for poultry (broilers) and assess the extent to which it can replace maize in broiler chicken diets.

The specific objectives were :-

a) To determine the chemical composition and biological value of three species of grain amaranth.

b) To determine the effect of feeding diets containing the three grain species in raw form on broiler chicken performance.

c) To assess the effect of different heat processing methods and fortification regimes on nutritive value of the grain.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 Background of Kenya's poultry and animal feed industries

2.1.1 The poultry industry

The Kenyan poultry industry has steadily grown at a rate of about 6% in the last decade. The current population consists of about 16 million indigenous and 7 million commercial (exotic) birds (MALDM, 1994). The commercial flock comprises 1.6 million layers and 5.4 million broilers and contributes about 50% of the eggs and 30% of the poultry meat produced locally (MALDM, 1994). Commercial poultry keeping is concentrated around urban areas mainly in Nairobi, Central and Coast Provinces. This is due to the availability of market outlets and easy access to inputs such as feed, drugs and day old chicks.

The local commercial flock is often characterised by low productivity. On the average, broiler chickens do not attain a liveweight of 2 kg at the market age of 8 weeks. Similarly, production of layers is about 240 eggs per bird per year (MALDM, 1989). The main constraints have been: Unreliable supply of high quality feeds and day old chicks; Substandard level of management and disease control; and lack of a well established market infrastructure for poultry and eggs.

The growth of the poultry industry is expected to

increase in future following the liberalisation of the day old chick and feed sectors. Other factors expected to favour this growth are:- i) increase in rural population; ii) fast growing urbanisation; iii) subdivision of existing farms into small units making them subeconomical for dairy or crop farming. This scenario is already being experienced in the heavily populated areas of Central Province; iv) the government's policy to remove duty on imported chicks, drugs and vaccines. Such intervention might favour the farmer through moderation of input prices. On the other hand, the government's move on privatisation of clinical and extension services might result in increased costs to the farmer. The prevailing market forces will determine the future growth of the industry.

2.1.2 The animal feed industry

The compounded animal feed industry in Kenya initially started with utilisation of milling by products such as wheat pollard, wheat bran, maize bran, maize germ and others (Magadi, 1994). With the increase in commercial livestock production, the demand for high quality feeds proportionally increased. To ensure the right quality, feed specifications were established under the Kenya Bureau of Standards (KBS, 1978) providing the much needed guidance to feed manufacturers. The trend has been a large increase in the use of whole cereal grains especially maize as raw materials for animal feed. This has created stiff competition between this

industry and feeding the increasing human population. With the latter receiving priority, the consequence has been intermittent supply of feed at high prices. Many manufacturers have failed to meet the stipulated KBS (1978) specifications, citing shortage of raw materials. The sale of substandard feeds in the market has resulted in low productivity especially in the case of non-ruminant animals.

The government policy is to encourage the use of local raw materials in compounded animal feed. In addition, the expected trend is towards increased support for production of oil seed crops and non conventional crops like sorghum and cassava (MALDM, 1994). Research will be encouraged to obtain crop ingredients that can be used in feed milling (MALDM, 1994). It is in this area that local production of grain amaranth can play a part through supply of both energy and protein requirements. However, the production and availability of this grain has been scarce. Its utilisation and nutritive value need to be established. This highlights the importance of research on this crop geared towards providing the necessary information.

2.2 Grain Amaranth (*Amaranthus* spp.)

Grain amaranth probably originated in Central and South America. The earliest archaeological record is that of *A. cruentus* species found in Mexico about 4000 BC, making it one of the oldest known food crops (Teutonico and Knorr, 1985). The grain was grown by the Aztecs and Incas during the pre-

Columbian times as a major food grain (NRC, 1984). The use of amaranth was discontinued after the Spanish conquest except for a few mountain areas in Mexico and the Andes. Pale seeded amaranth was also grown in Germany in the 16th century, India and Ceylon (Sri Lanka) in the 18th century and Interior China and Siberia in the late 19th Century (Teutonico and Knorr, 1985). Grain amaranth is grown widely in India as a subsistence crop (Jain *et al.*, 1977). The vegetable types of amaranth are important in Asia and Africa (NRC, 1984).

Kauffman (1992) documented some of the traditional food uses of grain amaranth. In Mexico, the grain is used to make alegria candies from popped seed while in India, it is mainly used to make confections known as ladoos. In Nepal, the flour is used to make gruel (sattoo) and pancake like chapatis. Kauffman (1992) further reported on new food uses such as baby foods in Mexico, extruded products in Guatemala and rolled flakes in Peru. In the United States of America, the grain is processed in combination with other grains to produce cold or hot breakfast cereals, breads, crackers and pastas. In Kenya, milled amaranth flour is added to maize based gruels (Kauffman, 1992).

In early nineteen seventies, interest in grain amaranth as one of the potential nutritionally balanced foods started at the Rodale Organic Gardening and Farming Research Centre (OGFRC) in the USA. This resulted in the initiation of amaranth studies at the centre and spurred similar efforts at various research centres (Kauffman, 1992). In Kenya, research

studies on grain amaranth started in 1984 in the department of crop science, University of Nairobi (Gupta & Thimba, 1992). These authors reported that grain amaranth could be used in Kenya, mainly as an ingredient in commonly consumed foods. The initial efforts to introduce the crop commercially to the Kenyan farmer were not followed up through extension work. Hence, the grain remains virtually unknown in many areas. Some farmers in Nanyuki, Kirinyaga and Meru districts of Kenya grow this grain but on limited acreage. However, vegetable amaranth is commonly eaten in most rural areas where it grows mainly as a weed.

2.2.1 Botanical characteristics and agronomical requirements

The Amaranthaceae family consists of hardy, fast growing herbaceous plants, which adopt readily to new environments (NRC, 1984). It belongs to the few non-grass plants which produce significant amounts of edible cereal-like grain. The family comprises of annual crops with brilliantly coloured leaves, stems and flowers. Some of the species are used as ornamentals all over the world (NRC, 1984).

The seedheads of grain amaranth resemble those of sorghum and can be as long as 50 cm. The small, ovular seeds occur in massive numbers on large terminal and lateral inflorescences. Seed colour ranges from white to black. The plant has a deep tap root system which makes it drought resistant and its broad leaf canopy effectively suppresses weed competition. The three major species used for grain production are *Amaranthus*

caudatus, *A. cruentus* and *A. hypochondriacus* (Kauffman, 1992).

Amaranth tolerates a wide range of soil types, from acid to alkaline and saline (Martineau, 1985). Many genotypes are adapted to the tropics. Amaranth does best at warm daily temperatures beyond 21°C (NRC, 1984). The crop is easy to establish, grows vigorously and is known to resist drought, heat and pests (NRC, 1984). The water requirements appear to be similar to those of sorghum. In the 1984 drought period in Kenya, amaranth and sorghum produced grain in areas where the maize crop failed due to inadequate rainfall (Gupta and Thimba, 1992). Among the species grown in Kenya, *A. hypochondriacus* species were early and medium maturing while *A. caudatus* and *A. cruentus* matured late. Maximum yields of 2580 kg/ha were reported in the crop research trials which compared favourably with those of other grain crops (Gupta and Thimba, 1992).

2.2.2 Chemical composition of the grain.

There is relatively higher protein and fat content in grain amaranth compared to cereal grains. The large variability in chemical composition appears to result from interactions among genetic make up, environmental factors and cultural practices (Bressani *et al.*, 1992). The crude protein content of the grain ranges between 12.5 and 19 % on dry matter basis comprising about 5% lysine and 4.4% sulphur amino acids (Teutonico and Knorr, 1985). Table 1 shows the essential amino acid content of amaranth grain, maize grain

Table 1 : Essential amino acid[1] content of amaranth grain, maize and soyabeans (% protein)

	Tryptophan	Methionine + Cystine	Threonine	Isoleucine	Valine	Lysine	Phenylalanine + Tyrosine	Leucine
A. cruentus (Mexican-1015)	1.5	4.6	3.0	3.2	3.8	5.0	6.6	5.0
A. hypochondriacus (Nepal-1024)	1.4	4.0	2.9	3.0	3.6	4.9	6.4	4.7
Maize grain	0.6	3.2	4.0	4.6	5.1	1.9	10.6	13.0
Soyabeans	1.4	3.1	3.9	5.4	5.3	6.3	8.1	7.7

Source: Senft (1980).

and soyabeans. Senft (1980) noted that amaranth protein complemented other grains in lysine and sulphur amino acids while other grains complemented amaranth in leucine content. Amaranth protein can efficiently supplement maize protein, especially in countries where maize is the main staple grain (Bressani *et al.*, 1992). The fat content of grain amaranth ranges from 5.4 to 17.0% on dry matter basis, containing about 50% linoleic acid (Teutonico and Knorr, 1985). In fourteen grain amaranth lines studied by Bressani *et al*, (1992), the protein content varied from 12.5% - 16% while fat content ranged from 7.7% to 12.8%. From the same study, the contents of various fatty acids as percent of total oil content were:- palmitic acid, 16.83-23.83%; stearic acid, 1.86 to 4.11%; oleic acid, 20.29 - 35.46%; linoleic acid, 38.25 - 57.86% (Bressani *et al.*, 1992).

Crude fibre levels range from 3.2 to about 8.5% (Cheeke and Bronson, 1980; Teutonico and Knorr, 1985). The starch content of pale seeded grain types was reported to range from 48 to about 62% (Teutonico and Knorr, 1985). The total mineral content of amaranth grain is generally higher than that observed in conventional grains. Compared with wheat, pale seeded grains contained about 3 times the quantity of calcium, 1.3 times the quantity of phosphorus, 2-4 times as much iron, 1.5 times as much copper and the same quantity of zinc as that in wheat (Pedersen *et al*, 1987). The black seeds were reported to be higher in calcium and phosphorus than the pale seeds (Pedersen *et al.*, 1987).

2.2.3 Nutritive value

The protein quality of amaranth grain has been evaluated using in vivo and in vitro methods. Bressani (1988) suggested that threonine was the most limiting amino acid. Carlsson (1980) and Betschart *et al* (1980) reported that leucine was the first limiting amino acid for humans followed by valine and threonine. Senft, (1980) noted that leucine, threonine, isoleucine and valine contents were two thirds to three quarters of the FAO/WHO recommended levels. Various authors reported different protein efficiency ratios (PER) for *A. hypochondriacus* in the range of 1.5-2.1 compared to that of 2.5 for casein (Betschart *et al.*, 1980; Sanchez-Marroquin *et al.*, 1980; Calderon *et al.*, 1985). Afolabi *et al.* (1981) found a high true protein digestibility (92%) but a surprisingly low biological value (46%) and a negative PER for *A. caudatus*. Results of the above studies indicated that grain amaranth was poorly digested and utilised and that *A. hypochondriacus* grain probably had the best nutritive value.

In a rat feeding study, low palatability and poor growth response was obtained with raw amaranth seeds (Cheeke and Bronson, 1980). Cooking improved the weight gain, suggesting the presence of heat labile toxic factors. The authors reported that phenolic compounds and saponins, both of which are astringent might have been the feed refusal factors causing the low palatability. However, they did not carry out analytical work to confirm the presence of these factors. *A. hypochondriacus* produced better results than *A. cruentus*.

(Cheeke and Bronson, 1980). Carlsson (1980) reported the presence of small amounts of phenolic content.

It is evident that the raw amaranth grain does not have biologically the protein quality suggested by its amino acid pattern (Bressani, 1988). This author reported that four selections from three species of the grain that were tested induced a low weight gain in rats which if expressed as protein quality would be only 65% of the value of casein. Some selections gave a better animal growth performance when fed raw than others. However, there was significant improvement in nutritive value upon an appropriate thermal processing of the grain. The difference that existed in the raw form largely disappeared so that all the selections gave essentially the same quality, close to that of the reference protein casein. The author indicated that the low animal performance from the raw grain was more due to inhibitory substances or nutrient unavailability rather than to essential amino acid pattern.

Pedersen *et al.* (1987) reported that trypsin and chymotrypsin inhibitor activities in grain amaranth were present at levels typical of cereal grains. Since the inhibitors were heat labile, their levels were greatly reduced by the heat processing methods applied. These authors further reported that the energy levels of the different amaranth products were generally higher than those of cereal grains due to the high fat content.

Takken and Connor (1986) studied the toxicological

effects of grain amaranth (*A. caudatus*) in pigs and found that feed refusal was initially high but declined as each experiment progressed. Adaptation to the diets took longer with increased amaranth level in the diet. In one experiment, 10 pigs died suddenly at various times over a 1 month period, beginning 7 weeks after the start of feeding. Nine of these had lesions of mild to severe myocardial degeneration with an accumulation of excess fluid in the body cavities. From these findings, the authors concluded that amaranth grain was toxic to pigs and that the steam press pelleting process was not effective for detoxification.

Mineral availability in *A. caudatus* was studied in balance experiments with growing rats (Pedersen *et al*, 1987). Results indicated that zinc availability was low as analysed in the rat femurs. The authors reported that femur zinc was a very sensitive indicator of zinc status in rats. The rats fed on the diets with the lowest levels of zinc were actually in negative zinc balance. These authors attributed the low zinc availability to the high phytate : zinc ratios. There were no apparent effects of processing on zinc availability. Calcium and phosphorus contents were lower in the bones of rats fed the unprocessed pale seeds compared to those fed the corresponding processed products. This was partly associated with reduced feed intakes among rats fed raw seeds (Pedersen *et al*, 1987).

The phytate content of amaranth was reported to be about 1.5 times that of wheat (Perdersen *et al*, 1987). Imeri *et al*.

(1985) suggested that phytates in amaranth may influence the bioavailability of zinc in test animals. Laovoravit *et al.* (1986) found raw amaranth contained 0.98 mg/kg of thiamin in the raw state and 0.5 mg/kg when autoclaved as compared to corn and wheat which contain 3.5 and 4.3 mg/kg thiamin respectively.

2.2.4 Performance of chickens fed grain amaranth diets

Various authors have conducted studies on the nutritional value of grain amaranth in chickens. Laovoravit *et al* (1986) fed day old broiler chicks for 17 days with diets containing 10 or 30% of either raw or autoclaved *A. cruentus* grain. A maize - soyabean meal control diet was used for comparison. Results showed that chicks on 30% raw grain had less ($P<0.05$) growth (318g/bird) than the control (377g/bird). In another experiment, the same authors fed chicks on diets containing 78.7% autoclaved amaranth. All the chicks lost weight by the 17th day and three of them exhibited symptoms of thiamin deficiency.

Waldroup *et al* (1985) fed broiler chicks up to 13 days of age on 20 and 40% raw or autoclaved *A. cruentus* and *A. hypochondriacus* grain diets. A control diet with no amaranth was used for comparison. Chicks on 20% raw *A. cruentus* or 20% autoclaved grain of either species had similar weight gains to those of the control group. Feed intake was slightly reduced at this level of inclusion. However, both weight gain and feed intake were significantly reduced at 40% inclusion, regardless

of the treatment. *A. hypochondriacus* caused a more severe reduction in performance than *A. cruentus* but this was partially alleviated by autoclaving. The findings were in contrast to those of Cheeke and Bronson (1980) who reported better rat performance from *A. hypochondriacus*.

Tillman and Waldroup (1986), incorporated autoclaved *A. cruentus* grain into broiler diets at 20 or 40% levels. The diet containing 20% amaranth grain autoclaved for 60 minutes gave similar chick weight gain (577g) at 21 days of age to that of the maize-soyabean meal control diet (578g). However, growth was significantly depressed at 40% level of inclusion regardless of the duration of autoclaving. In a second experiment, the same authors compared the nutritive value of autoclaved and extruded grain amaranth and found that extrusion resulted in better weight gains than autoclaving.

Acar *et al* (1988) fed chicks on diets containing 61.46 raw or autoclaved whole grain amaranth flour from 2 to 17 days of age. Chicks on autoclaved amaranth flour gave a similar performance to that of chicks on a maize-soyabean meal diet. Tillman and Waldroup (1988c) fed day old chicks on 0-50% extruded amaranth diets up to 49 days of age. There were no adverse effects on chick body weights, feed efficiency and carcass weight up to 40% inclusion.

Generally, the poor chick performance obtained by the different authors was attributed to toxic or feed refusal factors present in raw or insufficiently processed grain amaranth. However, in the feeding trials reported, no studies

were carried out to determine the nature or quantities of the suspected factors.

Some studies were conducted to determine the metabolisable energy (ME) level and amino acids availability of grain amaranth. Laovoravit *et al* (1986) reported gross energy, apparent and true metabolisable energy values of 3510, 2860 and 3,000 kcal/kg respectively of autoclaved *A. cruentus* grain. Tillman and Waldroup (1988a) reported an apparent ME value for extruded *A. cruentus* grain of 3,382 kcal/kg on as fed basis. The former authors used the quick feeding method of Vohra *et al* (1982) while the latter used a total collection method which gives similar results with that of using chromic oxide (Han *et al*, 1976). Tillman and Waldroup (1988b) using the same total collection method determined various amino acid availabilities. Dietary treatments of 0-50% amaranth were used and calculations were based on linear regression equations to estimate the values for 100% amaranth. Predicted availabilities for methionine, lysine and arginine were 85.00, 79.10 and 92.90% respectively. Laovaravit *et al* (1986) found that the growth of chicks fed autoclaved amaranth diets was equal to that of chicks to which lysine was supplied equally by corn protein and by free lysine. These authors concluded that lysine in amaranth was highly available.

2.3 Processing methods of feedstuffs and their effect on nutritive value

Various processing methods have been used to alleviate the negative effects of antinutritional factors on animals when plant feedstuffs are fed to livestock. Besides thermal treatment, chemical, physical and micro-biological methods have partly been effective in enhancing the nutritive value of such feeds. The material may be treated with acids, alkalis, organic solvents or saline solutions (Harris and Crampton, 1973; Calderon *et al*, 1985). Fermentation or treatment with enzymes such as phytase have been suitable for other feedstuffs (Oldfield, 1973; Pointillart, 1994). Some ingredients are steeped in warm or cold water, germinated or sun-cured. Long storage, grinding, rolling and dehulling have also been found effective for certain products (Harris and Crampton, 1973; Mendoza, 1973).

2.3.1 Thermal Processing

The removal of antinutritional factors in feedstuffs is very often the first reason for thermal treatment (Putier, 1993). Each thermal processing method presents an optimum between the ideal level for the animal organism and the beginning of too great a degradation of the protein and other nutrients that can hinder animal performance (Putier, 1993). Extrusion and pelleting are two important types of thermal processing used in preparation of animal feed.

Harris and Crampton (1973) defined the term extrusion as pushing of material through orifices of a die under pressure. The process improves ingredient utilisation and produces a nutritious product in an appetising form (Koeppe *et al*. 1987). During extrusion, the feedstock is put onto an endless screw consisting of several pressure rings of decreasing shape (Putier, 1993). This screw drives the product leading it to a small opening called a die. The temperature rise is caused by product friction between the screw and the jacket and by an increase in pressure. The pressure difference between the interior of the extruder and the exterior leads to a partial vaporisation of the water at the exit point and thus to an expansion of the product. The extrusion conditions such as temperature can be set according to the product required. According to Koeppe *et al*. (1987) the recommended temperature should be 120˚-150˚C to permit quick evaporation and product expansion. There is a decrease in product density with increasing temperatures. A measure of the extrudate diameter divided by the die diameter gives the so called 'Puff ratio'(Koeppe *et al*, 1987).

The development of extrusion processes over the years for both human and animal food and the different types of extruders are clearly described by Lusas and Riaz (1994). According to these authors, collet extruders are used for making puffed snacks from corn grit. The temperature of the ingredients is raised rapidly causing the starch to be dextrinised and partially gelatinised. The resulting mass

loses moisture and puffs immediately upon exit through a die to form a crisp, expanded curl or collet. Peisker (1994) reported that expansion of feed increased its intake. This was possibly due to activation of natural flavours and inactivation of appetite suppressant ingredients. In addition, the digestibility of such feeds increased due to:- inactivation of enzymes; gelatinisation of starch; release of natural fats from feed components giving better accessibility to digestive enzymes; modification of crude fibre structure making it more soluble and digestible (Peisker, 1994).

Extrusion normally involves much higher temperatures than pelleting. This process is therefore more destructive to vitamins and other sensitive feed additives, (Gadient, 1994). The vitamins that are most affected are A, B_1, E and folic acid. There should be sizeable fortification coverage for these vitamins in extruded feeds. The friction and temperature that builds up in an extruder is sufficient to destroy protease inhibitors (Cheeke and Shull, 1985; Lusas and Riaz, 1994).

Pelleting is a thermomechanical treatment which can be applied to any feed or raw material. The material is conveyed into a rotating die after addition of a low pressure steam, (Putier, 1993). The die contains numerous holes through which the product passes. It is pushed by rotating rolls situated inside the die. There have been contradictory reports on the nutritional effects of pelleting (Smits *et al.* 1993). Some researchers have reported improvement in digestibility and

feed conversion ratio while others have observed no effect. Smits *et al.*, (1993) found an increase in nitrogen digestibility when a legume seed based diet was fed to pigs. They associated the improvement in nutritive value to lowering of the antinutritional factor content in legume seeds by pelleting.

Early studies showed that pelleting mash feeds greatly increased their density, causing a marked growth improvement. This was because the chickens consumed sufficient amounts of the pelleted feed to meet their daily energy requirements (Scott, *et al.*, 1982). However, it was later found that birds could achieve the same by eating high energy density mashes. In diets containing underheated soya bean meal, Scott and associates reported that pelleting often resulted in a marked improvement in the feeding value, which they attributed to more complete destruction of trypsin inhibitors and other toxic factors.

Results of a study where maize, low tannin and high tannin sorghum were evaluated in mash or pelleted diets it was shown that pelleting improved weight gain and feed conversion efficiency regardless of the grain source (Douglas, 1990). However, pelleting did not decrease the assayable tannin in the high tannin sorghum. Pelleting was also reported to increase energy intake, growth rate and feed efficiency and had a higher beneficial effect at low than high energy level (Leclercq, 1986). Pelleting has however been shown to cause some destruction to certain feed additives but not by more

than 10-20% (Gadient, 1994). The most sensitive additives are vitamins A, C, carotenoids and antibiotics (Gadient, 1994).

2.3.2 Effect of processing methods on nutritive value of grain amaranth

Various treatment methods have been used to try and reduce (or remove) the growth inhibiting substances in raw amaranth grain. Heat processing methods have generally been most effective in enhancing the nutritive value. This is because the proteinaceous antinutritional factors such as trypsin inhibitors and lectins are inactivated by heat treatment. Although the exact nature of the inhibiting substances in amaranth are not yet known, it appears that they are mostly heat-labile.

Roasting, flaking, popping, wet cooking, autoclaving or extruding have produced improvement in nutritive value of grain amaranth (Laovoravit *et al*, 1986; Acar *et al*, 1988; Vohra *et al*, 1989; Bressani *et al*, 1992). Moist heat treatment appears to give the best results (Pond *et al*, 1991). Betschart *et al*. (1980), reported that the PER value and the protein digestibility of *A. cruentus* grain were unaffected by hot air popping. According to Bressani *et al*. (1992), wet cooking and extrusion exhibited the best protein quality with respect to weight gain and digestibility compared to popping, flaking and roasting. The same authors showed that atmospheric cooking and extrusion increased the true metabolisable energy in chicks compared to the raw grain.

Steam extrusion of grain amaranth appeared to be a more effective method of heat treatment compared to autoclaving based on weight gains and feed conversion efficiency values of broilers fed amaranth diets. (Tillman and Waldroup, 1986).

Thermal treatment can have positive but also negative consequences for the products (Bressani *et al*, 1992; Putier, 1993). The processing parameters of the different treatment conditions such as temperature, duration, moisture content, pressure and particle size influence the nutritional quality of the product (Huisman and Tolman, 1992; Putier, 1993). During heating, reducing carbohydrates may react with free amino groups of a protein, particularly the epsilon amino groups of lysine. Such carbohydrate-amino acid reactions (termed the 'Maillard' reaction) result in linkages that are not hydrolysed by digestive enzymes, making these nutrients unavailable to the animal (Scott *et al.*, 1982). Pedersen *et al.* (1987) found that roasting of *A. caudatus* grain decreased the amino acid content and lysine became the first limiting amino acid. Bressani *et al.* (1992) suggested that the amaranth grain should be processed under well controlled conditions in order to maintain its functional properties without causing losses in nutritive value.

Many studies have shown that there is a significant increase in food intake of cooked versus raw amaranth grain (Bressani *et al*, 1992). These authors suggested that the raw grain may present any of the following problems :- (i) lack of palatability; (ii) unavailability of amino acids (iii)

presence of antiphysiological or antinutritive substances. Pond *et al*. (1991) reported that the possible moist heat-sensitive growth inhibiting factors in grain amaranth were lectins, trypsin inhibitors or tannins.

2.4 Antinutritional factors (ANFs) and their effect on animal performance.

Nearly all sources of plant proteins possess associated factors which must be eliminated by special processing techniques to make them of maximum nutritional value (Scott *et al*. 1982). These antinutritional factors as they are called have a protective function in the plants and seeds but have negative effects on growth or health in man and animals (Huisman and Jansman, 1991; Huisman and Tolman, 1992). Some of the factors have a depressive effect on protein digestion and utilisation such as trypsin and chymotrypsin inhibitors, lectins, saponins and phenolic compounds. Others like phytic acid have a negative effect on the utilisation of minerals.

2.4.1 Protease inhibitors

This class of antinutritional factors consists of plant protein fractions with a molecular weight ranging from 4,000 to 25,000. The inhibitors in different plant species differ in their molecular weight, amino acid composition and physical structure but are able to inhibit the activity of proteolytic enzymes. They form stable, inactive complexes with proteolytic pancreatic enzymes, trypsin and chymotrypsin, thus

reducing the activity of these enzymes. This inactivation creates a negative feedback mechanism which stimulates the pancreas to secrete more enzymes. The enhanced secretion results in increased weight of the pancreas in small animal species due to hypertrophy and hyperplasia (Liener, 1980; Huisman and Jansman, 1991). Those animal species with a relative pancreatic weight exceeding 0.3% become hypertrophic while those with a weight below this value do not respond in terms of pancreatic hypertrophy (Cheeke and Shull, 1985; Huisman and Tolman, 1992). The interference with the digestive process and the related loss of endogenous protein result in growth depression. The increased trypsinogen output is very high in cystine thus increasing dietary methionine requirement (Cheeke and Shull, 1985; Van Kempen, 1993).

Applying heat has been the most successful method of degrading the proteinaceous antinutritional factors. Efficiency of the heat treatment depends on various factors as earlier mentioned. Dry heating is less effective in inactivating trypsin inhibitors than steam heating, autoclaving and extrusion (Huisman and Tolman, 1992). These authors showed that dry roasting inactivated 54 - 82% of the inhibitors while extrusion and autoclaving at 100°C for more than 15 minutes inactivated 78 - 98% and 85 - 100% respectively. Studies using raw soya beans showed depressed chick growth, feed utilisation and apparent retention of dietary dry matter and nitrogen and concurrent pancreatic hypertrophy (Herkelman *et al*, 1993). When the raw soya beans

were autoclaved for 20 minutes at 110°C, trypsin inhibitor activity was reduced. This resulted in improved chick performance and reduced pancreatic weight (Herkelman *et al*, 1993).

Koeppe *et al*. (1985) isolated trypsin inhibitors from *A. hypochondriacus* seeds. The inhibitor preparation was very thermostable retaining 20% of its original activity after 7 hours of heating at 100°C. These authors reported higher levels of trypsin inhibitor activity in amaranth seed (0.52 trypsin inhibitor units (TIU/mg) than corn (0.35 TIU/mg) and wheat (0.10 TIU/mg). Koeppe *et al*. (1987) reported reduction of trypsin inhibitor level from 0.52 TIU/mg to 0.20 TIU/mg after extrusion at 150°C of an amaranth-maize gluten meal (80:20) mixture.

Calderon *et al*. (1985) reported an inhibition level of 3.3 TIU/mg of protein in *A. hypochondriacus* crude extract. However, Pedersen *et al* (1987) suggested that trypsin and chymotrypsin inhibitor activities in amaranth were typical of those found in cereal grains. Their levels were greatly reduced by the heat processing methods applied. Liener (1980) reported that most of the plant protease inhibitors are destroyed by heat, an effect generally accompanied by enhancement of the nutritive value of the protein.

2.4.2 Tannins

Tannins are polyphenolic compounds which precipitate proteins from aqueous solution (Cheeke and Shull, 1985).

Depending on the structural type, they are classified as hydrolysable or condensed tannins. They have a strong hydrogen-bond affinity to the carboxyl oxygen of the peptide group of proteins. The bound proteins become insoluble and indigestible (Lorenz and Wright, 1984; Huisman and Tolman, 1992). Tannins may form complexes with digestive enzymes resulting in a decreased enzyme activity and hence decreased nutrient digestibility. Tannins form complexes with carbohydrates but not as easily as with proteins (Huisman and Tolman 1992). Other effects include reduction of mineral bioavailability, damage to the intestinal mucosa and toxity after absorption (Lorenz and Wright, 1984; Huisman and Tolman, 1992). Generally, tannins interfere with different aspects of the digestive process resulting in reduced growth and reduced feed conversion efficiency. They also cause lower palatability due to their astringent taste (Huisman and Tolman, 1992). The levels of tannin reported in grain amaranth are 0.043-0.116 catechin equivalent (Teutonico and Dietrich, 1985; Lorenz and Wright, 1984). Such levels are small in comparison with those in sorghum and millet, which can have as much as 3% tannins (Lorenz and Wright, 1984).

The dark seeded varieties of grain amaranth have been reported to have higher tannin content than the light coloured ones (Lorenz and Wright, 1984). Carlson (1980) reported the presence of small amounts of phenolics (0.02 - 0.25%) in grains of different amaranth species and found negative correlation between weight gain and phenolic content. Tillman

and Waldroup (1986) suggested that since phenolics are heat labile, they might be the primary cause for any observed toxic or feed refusal factors in raw amaranth grain. According to Becker *et al.* (1981) and Pedersen *et al.* (1987), tannins occur in minor amounts in grain amaranth at levels typical of other cereal grains. Tannins are concentrated in the testa and dehulling decreased their content (Lorenz and Wright, 1984; Pedersen *et al*, 1987). Imeri *et al* (1985) found that the small tannin content of grain amaranth disappeared after 30 minutes of autoclaving.

2.4.3 Hemagglutinins (Lectins)

Hemagglutinins also refered to as lectins are proteins which are mostly present in plants in the form of glycoproteins (Cheeke and Shull, 1985; Huisman and Tolman, 1992). They cause the clumping or agglutination of red blood cells in vitro. They have a high affinity for sugar molecules. Their primary effect is binding the mucosa of the intestinal wall (Huisman and Tolman, 1992). The damage to the intestinal epithelial cells can result in:- decreased absorption of nutrients; a change in the activity of brush border enzymes and hypersecretion of endogenous protein due to shedding of damaged cells; increased production of mucins and loss of plasma proteins to the intestinal lumen (Cheeke and Shull, 1985; Huisman and Tolman 1992). These adverse effects result in reduced growth. Liener (1980) reported that animals fed a raw field bean diet containing lectins developed liver

necrosis and had diminished levels of several liver enzymes. Lectins can be transported into the systemic circulation and cause production of specific lectin antibodies of IgG class (Huisman and Tolman, 1992). There was evidence that lectins impair the immune system leading to greater sensitivity to bacterial infection (Cheeke and Shull, 1985). Changes in gut permeability may lead to invasion by normally innocuous intestinal microflora such as Escherichia coli. Lectins are destroyed by moist heat (Cheeke and Shull, 1985).

Koeppe and Rupnow (1988) reported lectin levels of 0.308, 0.323 and 0.352 mg per mg protein in *A. hypochondriacus*, *A. cruentus* and *A. caudatus* respectively. The same authors purified a lectin from *A. cruentus* seeds using affinity chromatography and found it to be relatively heat labile, retaining about 10% of its original activity after heating for about 5 minutes at 70°C and after just 1 minute at 100°C. Calderon *et al.* (1985) extracted hemagglutinins from *A. hypochondriacus* seed using a saline buffer. There was significantly higher protein efficiency ratio (PER) and net protein ratio (NPR) for the saline extracted amaranth meal than for the whole meal. Similarly, Kauffman (1992) reported that the protein efficiency ratio of amaranth flour was improved by removal of lectins through heat processing.

2.4.5 Saponins

Cheeke and Bronson (1980) suggested that phenolic compounds and saponins might be the feed refusal factors in

raw grain amaranth. Since both factors have an astringent taste, they could cause low palatability.

Saponins are glycosides characterised by their bitter taste, foaming properties in aqueous solution and ability to haemolyse red blood cells (Huisman and Tolman, 1992). Saponins can be considered as less important because levels are low in most common feed ingredients for monogastric animals (Huisman and Tolman, 1992). These authors have however, reported that poultry are much more sensitive to saponins than other monogastric animals and ruminants. Saponins are inactivated by proper heat processing (Liener, 1969).

2.4.5 Phytic acid

Phytic acid is a cyclohexane compound with 6 phosphate groups (inositol phosphate). This phosphate (phytin phosphate) is largely unavailable to non-ruminant animals (Huisman and Tolman, 1992). In feeds of plant origin, two thirds of phosphorous is present as phytin phosphate which has a low digestibility. Phytic acid inhibits a number of digestive enzymes such as pepsin, pancreatin and a-amylase (Huisman and Tolman, 1992). Its interaction with protein and calcium ions was also found to decrease protein solubility (Maga, 1982). Of further nutritional significance is that phytic acid can chelate or form complexes with various minerals, producing phytates. This results in reduced availability of Ca, Mg, Zn, Cu and Fe (Cheeke and Shull, 1985;

Huisman & Tolman, 1992). The decreasing order of stability of phytate-mineral complexes is:- zinc, cobalt, magnesium and calcium. In mature cereal grains, 60-80% of the total phosphorus is bound as phytic acid and 50-60% in soya beans. Phytates are fairly stable to heat and autoclaving although a 70% loss in soya phytate was reported after 2 hours of autoclaving (Maga, 1982). Fermentation liberates phosphates from phytic acid (Cheeke and Shull, 1985) while addition of the enzyme phytase increases the digestibility of phosphorus considerably (Huisman and Tolman, 1992).

Phytic acid in practical diets increases the zinc requirement. Natural and synthetic chelating agents can markedly improve the utilisation of zinc and prevent the deleterious effect of the binding of zinc by phytic acid (Scott *et al*, 1982). The high stability constant for ethylenediaminetetraacetate (EDTA) in chelating metal ions enhances availability of zinc, manganese and copper to chicks. This is because EDTA acts as a carrier of the polyvalent cations thus availing them for metabolism (Scott *et al*, 1982). O'dell *et al*. (1964) found addition of 0.5% phytic acid caused a marked growth depression in chicks and other symptoms typical of zinc deficiency. Significantly, the addition of 0.1 % EDTA supported a growth rate approaching that of the control and eliminated all gross symptoms of the deficiency (O'dell *et al*. 1964).

The phytate content of amaranth varies with the species but generally falls within the range of 0.27-0.62% (Lorenz and

Wright, 1984; Teutonico and Knorr, 1985; Pond *et al*, 1991). Phytate is distributed throughout kernels of amaranth and cannot be reduced by removal of the seed coat (Lorenz and Wright, 1984). High phytate : zinc ratios in amaranth have been associated with low zinc availability in rat diets (Pedersen *et al*, 1987).

2.4.6 Threshold levels of ANFs in animal nutrition.

There is little indication about threshold levels of ANFs that can be tolerated without causing negative effects on animal performance (Huisman and Tolman, 1992). Information on such levels is important for nutritionists and plant breeders. The effect of ANFs is dependent on the species, age or liveweight of the animal. It has been indicated that tannin levels of upto 3g/kg diet can be tolerated. Lectins from certain plants are more toxic than others. Adequate nutritional evaluation of ANF-containing seeds and the determination of threshold levels are hampered by lack of analytical techniques (Huisman and Jansman, 1991). There exists no known minimal admissible quantities of ANFs and therefore the animal is the only indicator (Putier, 1993). A systematic determination of antinutritional quantities has still to be found. Huisman and Jansman (1991) concluded that there is need for more research and information on threshold levels which can be used in feed formulations.

2.4.7 Assessment of tissue damage due to ANFs

After ingesting toxic plant materials, numerous metabolic processes occur before the toxicant involved exerts its toxic effect or is excreted (Cheeke and Shull,1985). These processes may occur in the gastro intestinal tract, in the liver or in other tissues. Liver tissue has a very high level of toxin metabolising enzymes which detoxify the absorbed materials. Various procedures are used to assess pathological changes when animals are fed toxic materials. Such procedures or techniques depend on what tissues are affected by the particular toxicant. In the case of hepatotoxins, liver biopsies can be used to assess sequential changes in liver histology (Cheeke and Shull, 1985). Tissue damage and certain intoxicants are among some of the factors that induce increased leucopoiesis, causing the release of more than the normal number of white cells into the circulation (Jennings, 1970).

2.4.8 Analysis of ANFs

There is lack of specific adequate analytical methods for ANFs (Huisman and Tolman,1992). There are various methods to determine the levels of tannins and other (poly)phenols but none of these distinguishes between toxic and non-toxic polyphenols. Both colorimetric and gravimetric methods are used for quantitation of tannins (Makkar *et al*, 1993). The colorimetric method commonly used is the HCl-vannillin method of Burns (1963) and modified by others such as Price *et al*

(1978). The standard trypsin inhibitor assay is based on the determination in soya. It is uncertain whether this assay can be used without modification to determine the trypsin inhibitor in other seeds (Huisman and Tolman, 1992). Bovine trypsin is used in this assay even when these products are fed to pigs and poultry. Different analytical methods have been employed and the units in which trypsin inhibitor activity is expressed often differ. It is therefore difficult to compare results obtained in different laboratories (Huisman and Tolman, 1992). The method of Kakade *et al*. (1974) on soya beans was used by Koeppe *et al* (1985 and 1987) on amaranth to provide comparable results.

In vitro agglutination of red blood cells is used to detect and quantify lectins. However, there are differences in agglutination activity measured with erythrocytes of different animal species (Huisman and Tolman, 1992). Calderon *et al*. (1985) used human erythrocytes to test the agglutination capacity of hemagglutinins in amaranth. There are findings showing that hemagglutination method is not sufficiently specific (Huisman and Tolman, 1992). There are no standardised methods for determining the phytate content of feedstuffs. Quantitative determination of phytic acid is based on the analysis of phosphorus or iron in the isolated ferric phytate. It can also indirectly be determined from the residual iron in solution after the precipitation of ferric phytate from a known concentration of ferric salt in acid solution (Maga 1982).

CHAPTER 3

3.0 CHEMICAL AND BIOLOGICAL EVALUATION OF THREE SPECIES OF RAW GRAIN AMARANTH

3.1 Introduction

The nutritive value of grain amaranth can be determined mainly through feeding and assessing broiler chicken performance. Before carrying out such feeding trials, it is important to investigate the chemical composition and biological value of the grain as an indicator of nutritive value. Such chemical composition evaluation will indicate levels of proximate fractions, mineral content, amino acid profiles as well as the level of antinutritional factors. Biological determination of energy content (metabolisable energy) will add onto chemical composition. Information on chemical composition will be used in formulating diets used in subsequent feeding trials.

3.2 Objective

To determine the chemical composition and biological value of three species of grain amaranth.

3.3 Materials and methods

3.3.1 Source and species of grain amaranth

Grain amaranth used in this study was obtained from the Nanyuki based Amaranth and Natural Foods Company. Nanyuki is

a semi-arid area located near the slopes of Mount Kenya on the leeward side.

Three grain species were chosen for analysis, namely *A. cruentus*, *A. hypochondriacus* and *A. caudatus*. These species have light coloured grain. The varieties selected within each species depended on availability. However, more varieties from *A. hypochondriacus* were tested since this species has been found to perform well in many locations in Kenya (Gupta and Thimba, 1992). For *A. hypochondriacus*, three varieties, namely 1023, 1024 and Jumla were evaluated. However, in *A. cruentus* and *A. caudatus*, only one variety of each species, 434 and 1113 respectively were analysed. The variety Jumla was reported to have originated from the Jumla region of Nepal while the other varieties corresponded with the Rodale Research Centre (see literature) collection.

3.3.2 Chemical and biological assays

a) Proximate analyses

Grain samples from the above 5 varieties of amaranth were finely ground using a Wiley mill with a 1mm sieve and stored in well sealed containers. The standard procedures (AOAC, 1984) were used in determining the various proximate fractions. All the analyses were carried out in duplicate.

In determining moisture content, two grams of sample were weighed into an aluminium dish of a known weight. The dish was placed in an oven at 105°C and heated for about four hours. It was then cooled in a dessicator for thirty minutes

and reweighed. The moisture content was calculated as the difference between the initial and the final weight. Ash content in the sample was determined as follows:- two grams of sample were put into a silica dish and the weight recorded. The dish was placed in a muffle furnace at 600°C for four hours, removed from the furnace, allowed to cool, transferred to a dessicator and reweighed. The difference between the two weights represented the ash content of the sample.

In determining the ether extract, two grams of the sample were transferred into a paper Soxhlet thimble and plugged with a little cotton wool. The thimble was placed into the barrel of a Soxhlet extractor and a previously weighed 150 ml flat bottomed flask attached. The extractor was filled with diethyl ether which was allowed to siphon into the flask until half full. A reflux condenser was attached and the cooling water turned on. The extraction was allowed to continue for 12 hours. The thimbles were then removed and the ether in the barrel of the extractor poured into the collecting flask. The flasks were transferred to a drying oven at 105°C for about two hours. After cooling in a dessicator the flasks were weighed. The weight gain represented the amount of lipid in the sample.

Crude fibre was determined as follows:- two grams of sample were transferred into a 600ml beaker and boiled with dilute sulphuric acid (25ml of 2N H_2SO_4 made up to 200 ml with hot water) for 30 minutes on a hot plate, then filtered using filter sticks packed with glasswool. The residue was washed

three times with boiling water, then boiled with 25ml of 1.78N KOH made up to 200ml with hot water. This continued for another thirty minutes after which the beaker contents were filtered and washed as before. The residue was finally washed and filtered three times with small amounts of ethanol. The glass wool plus fibre residue was transferred to a silica dish, dried in an oven at 105°C for two hours, cooled and weighed. The dish was thereafter ignited in a muffle furnace at 600°C, cooled and reweighed. The difference between the dish weights before and after ignition represented the crude fibre content of the original sample.

The crude protein content was determined as follows:-

One gram of the sample was weighed into a kjeldahl flask. Half a selenium tablet was added to act as a catalyst. While placed in the fume cupboard, 10ml of conc. H_2SO_4 was dispensed into the flask. The mixture was boiled until colour free, cooled and then carefully transferred into a distillation flask. The excess acid was neutralised with 40ml of 50% NaOH and the mixture steam distilled in a Hoskins apparatus. The liberated ammonia was trapped in 25 ml of boric acid solution. Following the distillation, the boric acid was titrated against 0.1N HCl to return the pH to the original value (a grey-red endpoint). The titration value was recorded. The nitrogen content of the sample was calculated based on the fact that 1 litre of N HCl represented 14 grams of nitrogen. The amount of nitrogen obtained was then multiplied by a

factor of 6.25 to give the amount of crude protein in the sample.

b) Determination of mineral content

Except for phosphorus, the minerals were determined by Atomic Absorption Spectroscopy (Perkin-Elmer, model 2380). Standard procedures (AOAC, 1984) were used for sample preparation while the manual for Perkin-Elmer equipment provided the specific analytical methods.

The sample preparation was carried out in duplicate. One gram of finely ground sample was placed in a 100 ml Kjeldahl flask. A digesting mixture was added consisting of 15 ml 70% perchloric acid and 5 ml concentrated nitric acid. The mixture was heated for several hours until it became clear. On cooling, it was carefully transferred to a 100ml volumetric flask and filled to the mark with deionised water. For calcium determination, 1% lanthanum oxide was added to remove interference from the phosphate ions. From the stock solution made, 5 ml was pipetted and fed into the Perkin Elmer Flame Spectrophotometer for determination. An oxyacetylene flame was used to convert the solution into an atomised vapour. The corresponding lamp for the test mineral was used to beam light at a specified wavelength at which that mineral absorbed maximally. The absorbency varied proportionally with the concentration of the test element. Based on a standard curve, the concentration of the test element was calculated internally to give a direct reading. If the reading was

outside the linear range of the standard curve, further dilution was done before repeating the measurement. Depending on the dilution, the concentration of the test element in the sample was calculated.

A colorimetric method was used for phosphorus determination. The feed sample (in duplicate) was digested and diluted to 100ml as described for other minerals above. A known amount of the liquid sample was then added to 15ml of a mixed solution of diluted (1:2) nitric acid, ammonium metavanadate solution (2.5g/litre) and ammonium molybdate solution (50g/litre) in a ratio of 1:1:1. The mixture was made to 50ml with deionised water. A yellow/orange colour developed from the reaction with phosphorus. The colour intensity of the samples and the standards was measured using a Beckman Model DU-8B spectrophotometer at 450nm wavelength. The standard solutions were prepared from potassium hydrogen phosphate dissolved in deionised water. To get a concentration of 1000 ppm, 4.3871g of KH_2PO_4 were dissolved in one litre of water. Based on this, standard concentrations of 0-40 ppm were made at 5 ppm intervals and their absorbance measured. A standard curve of absorbance versus concentration was made and used for calculation of phosphorus content in the samples.

c) Gross and metabolisable energy determination

Samples of raw and heat processed grain amaranth were assayed in triplicate for their gross and true

metabolisable energy (TME). The method of Sibbald (1976) as updated by the same author in 1986 was used. This method involves precision feeding which ensures that a known quantity enters the alimentary canal of a bird at a known time. The procedure avoids the need to recover waste feed, prevents feed selection and eliminates variations of intake among birds.

For each sample, 3 adult Isa brown cockerels were starved for 24 hours to empty the alimentary canal. Each bird was then fed with 40g of the milled sample using a stainless steel tube funnel and plunger device designed for this purpose. The stem of the funnel was carefully inserted from the open beak down the oesophagus into the crop. The feed was poured into the funnel and pushed direct into the crop using the plunger. The funnel was then slowly withdrawn using a rotary motion and applying gentle pressure to the oesophagus to ensure removal of any feed particles sticking to the funnel stem.

After feeding, each bird was placed in a wire cage and an excreta collection tray placed underneath. The birds were put in alternate cages to avoid cross contamination of the feacal material. Each collection tray was larger than the bottom of each cage in order to reduce the chance of excreta loss. Water was provided *ad libitum*. Three other birds similarly fasted were not fed, but remained fasted to serve as negative controls. The excreta collected from them provided an estimation of the metabolic and endogenous losses. The excreta voided by each bird for the next 24 hours was collected, dried in an oven at 60°C and allowed to cool.

Weight of the dry excreta was recorded. Samples of the excreta from the fed and unfed birds were assayed for gross energy using an adiabatic bomb calorimeter, Model IKA-C400. Calculation of apparent and true metabolisable energy was based on the following equations by Sibbald (1989):-

$$\text{AME/g of feed} = \frac{(F_i \times GE_f) - (E \times GE_e)}{F_i}$$

$$\text{TME/g of feed} = \frac{[(F_i \times GE_f) - (E \times GE_e)] + (FE_m + UE_e)}{F_i}$$

Where:-

F_i = feed intake (g)

E = excreta output (g)

GE_f = gross energy/g of feed

GE_e = gross energy/g of excreta of fed birds

$(FE_m + UE_e)$ = gross energy/g of excreta of unfed birds

FE_m = metabolic faecal energy

UE_e = endogenous urinary energy

d) Determination of tannin content

The above determination was based on the method for analysing tannins in sorghum grain by Price *et al.* (1978), a modified procedure of the original HCl-Vanillin assay of Burns (1971).

Each sample was ground to a 1 mm particle size and analysed in duplicate. One gram of sample was weighed into a 50ml conical flask. Fifty millilitres of absolute methanol

was added and the flask plugged with a cork stopper. The mixture was thoroughly shaken at first and thereafter occasionally for a few hours. The flasks were left to stand for 22 hours at room temperature for sample extraction. Before analysing the sample extracts, the following reagents were prepared:-

(i) 2% Vanillin - 4% HCL in distilled methanol. This was obtained by adding 4% vanillin in distilled methanol (w:v) to an equal volume of 8% HCl in methanol (v:v). (ii) 4% HCl in methanol was prepared by adding equal volumes of 8% HCl and pure methanol. This reagent was used for individual blanks.

For the analytical procedure, 1 ml of the sample extract was pipetted into its set of duplicate test tubes and capped with plastic stoppers. One ml of methanol was pipetted for the general blank. To one test tube of each sample and to the general blank, 5 ml of 2% vanillin - 4% HCl was added at 30 second intervals. To the second test tube of each sample, 5ml of 4% HCl was added at 30 second intervals to become the sample blank. Reaction time allowed for each test tube was 20 minutes. The general blank was used to zero the spectrophotometer (Beckman model DU-8B). Starting at 20 minutes, the optical density of each sample was measured at 500nm in the same order as reagent addition, at 30 second intervals.

The standard solutions were prepared as follows:- A stock solution of 200mg catechin in 200ml absolute methanol was first prepared. From this, concentrations of 0.05, 0.10,

0.25 and 0.50 mg/ml were made by adding respectively 5, 10, 25 and 50ml of stock solution to 100ml volumetric flasks and making up to volume with methanol. Each of the 4 standard solutions was pipetted (1 ml) into its duplicate set of test tubes and subjected to similar treatment described for the sample extracts above. A standard curve was prepared by plotting the average absorbance readings of the standard solutions against the catechin concentrations in mg/ml. The slope of the curve was determined. Catechin equivalent values for the unknowns were calculated using the formula:-

$$\frac{5}{\text{Slope}} \times \text{average absorbance of unknown} = \text{Catechin Equivalents (\%)}$$

The value 5 eliminates the steps to convert mg of catechin/ml to percent catechin equivalents. It is assumed that 1% catechin is equivalent to 1% tannin. The use of catechin equivalents has been reported to overestimate tannin content (Price *et al.*, 1978). The use of sample blanks corrects for the absorbance of non-tannin components (pigments) in the extracts. The difference in absorbance readings of the sample and the sample blank is used as the absorbance of the unknown (sample) in the above formula.

e) Determination of trypsin inhibitor content

The method followed in this assay was that of Kakade *et al.* (1974) used in determination of trypsin inhibitor activity of soya products. These authors reported that this method was more accurate and reproducible than that of Kakade (1969) and

was particularly suitable for the heat processed soya samples. It involves extraction of the inhibitors from the sample and mixing the suspension with bovine trypsin. The activity of the remaining trypsin is measured by adding benzoyl-DL-arginine-P-nitroanilide (BAPNA) as a substrate under standard conditions. The p-nitroaniline released is measured spectrophotometrically. From this, the amount of pure trypsin inhibited per unit weight of sample can be calculated.

The reagents and other materials used were as follows:-

i) Tris buffer:- Tris (0.05M, pH 8.2) containing 0.02M $CaCl_2$. Tris (hydroxymethyl) methylamine (6.05g) and $CaCl_2.{}_2H_2O$ (2.94g) were dissolved in 900ml deionised water. The pH was adjusted to 8.2 with hydrochloric acid and the volume made to 1 litre with distilled water.

ii) BAPNA substrate:- Benzoyl - DL-arginine-p-nitroanilide (BAPNA) hydrochloride (40mg) was dissolved in 1 ml dimethyl sulphoxide and diluted to 100ml with tris buffer previously warmed to 37°C.

iii) Trypsin solution was made by dissolving crystalline bovine trypsin (20mg) in 1 litre of 0.001M HCl.

iv) Sample extracts:- Samples of grain amaranth, raw soya bean and soya bean meal were analysed. One gram of finely ground sample was extracted by adding 50 ml of 0.01M NaOH. The pH of the resulting suspension was adjusted to between 9.5-9.8 with 1M NaOH or 1M HCl. The suspension

was constantly shaken and then left to stay overnight in the fridge (4°C).

The analytical procedure was as follows:-

One ml of each sample suspension was pipetted into a triplicate set of test tubes and adjusted to 2ml with distilled water. In a second set of test tubes, 2ml of water was put without any sample extract. After adding 2ml of trypsin solution to each, the test tubes were placed in a water bath at 37°C. To each tube, 5ml of BAPA solution previously warmed to 37°C was added. Exactly 10 minutes later, the reaction was terminated by adding 1ml of 30% acetic acid. After thorough mixing, the contents of each tube were filtered and the absorbance of the filtrate measured at 410nm against a reagent blank. The blank was prepared by adding 1ml of 30% acetic acid to a test tube containing trypsin solution and water (2ml each) and then adding 5ml of BAPA solution. The difference in absorbance readings between the two sets of test tubes (with or without sample) reflected the trypsin inhibitor activity. One trypsin inhibitor unit (TIU) was that which caused 10% inhibition of trypsin in 10 minutes under the described assay conditions.

f) Determination of amino acid profiles

The method used for separation and quantitative determination of amino acids was ion exchange chromatography. This automated method was first described by Spackman *et al.* (1958) and thereafter improved by various scientists. The

following determination was done in accordance with the manufacturers manuals, Pharmacia instruction manual (1988) and handbook for amino acid analysis, (1988).

In the amino acid analyser the separation column is packed with a resin having a negative charge. The amino acids are loaded at a low pH which ensures they are all positively charged. The conditions of the column are then altered to increase the pH, the temperature and the concentration of the buffer ions. These changes result in the isoelectric point of an amino acid being reached. At this point, the ionic attraction to the resin is lost and the amino acid elutes from the column. The conditions are adjusted so that the iso-electric points for the different amino acids are reached at different times which effects the separation.

i) Sample preparation

Each sample was weighed in quadruplicate: - One duplicate for direct acid hydrolysis and the other one for oxidation, prior to hydrolysis. Depending on the nitrogen content of the sample, the weights ranged from 0.5g to 1gm.

ii) Acid hydrolysis

The samples were weighed into 50ml pyrex glass bottles followed by addition of 25 ml of 6M HCl. They were then heated in an oven at 110°C for 24 hours. The bottle tops were loosely placed at the start and then screwed tightly after one hour of heating. This was to ensure removal of oxygen. Each

sample on cooling, was partly neutralised with about 20ml of 7.5N NaOH. It was then carefully transferred into a 100ml volumetric flask whereby the pH was accurately adjusted to 2.2. A sodium citrate buffer at pH 2.2 was then added upto the mark. A portion of the mixture was filtered using a 0.2mm membrane (millipore) filter. The filtrate was put in a labelled vial and put in the fridge awaiting amino acid analysis.

iii) Oxidation Procedure

Direct acid hydrolysis causes destruction of sulphur amino acids. Feed samples are oxidised prior to acid hydrolysis to allow accurate quantitation of cysteine and cystine as cysteic acid and methionine as methionine sulphone. About 5ml of performic acid was used for the oxidation per sample consisting of 4.5ml 88% formic acid, 0.5 ml 30% H_2O_2 and 25mg phenol. The samples were weighed in 50ml glass bottles as described above. After adding the oxidation mixture, a magnetic capsule was added followed by thorough mixing. The samples were then placed in ice and put in the fridge for 24 hours. The oxidation reaction was terminated by adding 0.8g of sodium metabisulphite to each sample. This was followed by adding 25ml of 6M HCl and the procedure continued as described above for acid hydrolysis.

iv) Analysis of amino acids

From the vial, 30 ul sample was pipetted into a marked capsule and placed in the autoloader compartment of the analyser. It was then automatically fed and loaded onto a separating column containing a cation exchange resin. Sodium citrate buffers were then pumped through the column to separate the various amino acids. In addition to the loading buffer (pH 2.2), four buffers of increasing pH (2.65, 3.35, 4.35 and 8.6) were used in sequence. The column temperature was accurately controlled between 60 and 80°C for specified time periods. The column eluate was then mixed with ninhydrin reagent and the mixture passed on to the high temperature reaction coil. Ninhydrin reacted with the amino acids in the eluate to form coloured compounds. The mixture was then fed to the photometer unit where the intensity of the coloured compound was determined by measuring the absorbance at 440nm wavelength for imino acids and 570nm for all the other amino acids. The photometer output was connected to a chart recorder where the amino acid concentrations were recorded as a series of peaks. The retention time of the peak on the chromatogram identified the amino acid while the area under the peak indicated the quantity. A calibration analysis using standard (pure) amino acids was performed before each series of analysis to produce a standard chart for comparison and form the basis for the quantitative calculations.

3.4 Results and discussion

3.4.1 Proximate analyses and mineral composition

The proximate composition and levels of various minerals in different varieties of grain amaranth are presented in Table 2. The mean crude protein content of *A. hypochondriacus*, *A. cruentus* and *A. caudatus* species was 15.42, 15.96 and 16.85% while the fat content was 6.72, 6.98 and 7.82% respectively. The respective crude fibre contents for the three species was about 6.73, 7.95 and 8.90% while the mean ash content was 3.04, 3.17 and 3.44% respectively. There were minor differences in proximate composition between *A. hypochondriacus* and *A.cruentus* species.

On the whole *A. caudatus* had the highest crude protein, crude fibre, fat and ash levels while *A. hypochondriacus* spp. had the lowest. Amongst the latter species, the variety Jumla had slightly higher proximate fractions compared to the varieties 1023 and 1024.

The mean calcium, phosphorus, magnesium, zinc, and manganese levels for the three species were 0.19%, 0.78%, 0.50%, 30.40 ppm and 38.00 ppm, respectively. The mineral levels were generally highest in *A. caudatus* and lowest in *A. hypochondriacus* spp. This was the same trend for proximate composition. Again, the variety Jumla had a higher mineral content than other *A. hypochondriacus* varieties (1023 and 1024).

Table 2: Proximate analyses and mineral composition of 3 species of grain amaranth[1]

Species	A.hypochondriacus			A.cruentus	A.caudatus	
Variety	1023	1024	Jumla	434	1113	Mean
Dry matter, %	91.62	91.88	91.32	91.07	89.90	91.16
Crude protein, %	15.39	15.28	15.60	15.96	16.85	15.82
Ether extract, %	6.68	6.73	6.77	6.98	7.82	7.00
Crude fibre, %	6.65	6.10	7.45	7.95	8.90	7.41
Ash, %	2.98	2.85	3.28	3.17	3.44	3.14
Ca, %	0.17	0.19	0.22	0.15	0.20	0.19
Mg, %	0.47	0.46	0.50	0.53	0.52	0.50
P, %	0.78	0.71	0.77	0.75	0.89	0.78
Fe, %	0.03	0.02	0.02	0.05	0.04	0.03
K, %	0.51	0.52	0.51	0.52	0.61	0.53
Na, ppm	70.00	74.00	78.00	82.00	91.00	79.00
Mn, ppm	32.00	42.00	39.00	37.00	40.00	38.00
Cu, ppm	3.00	4.00	4.00	4.00	5.00	4.00
Zn, ppm	26.00	31.00	26.00	30.00	39.00	30.40

[1]Air dry basis

The proximate composition values were within the range reported by other authors (Teutonico and Knorr, 1985; Pedersen *et al*, 1987). Bressani *et al*., 1992 reported levels of fat (up to 12.8%) in 14 lines from *A. hypochondriacus*, *A. caudatus*, *A. cruentus* and *A. hybridus* while Pond *et al*. (1991) reported high levels of ash, up to 17.4% in black seeds of *A. hypochondriacus*. Amaranth grain has a higher crude fibre than maize grain which only contains 2-3% (Appendix 1). The high fibre content of amaranth can adversely affect digestibility and metabolisable energy of the grain. On the other hand, grain amaranth has a higher fat content than maize which goes to boost its metabolisable energy content. The net effect is a similarity in energy values for both grains (Appendix 1). The protein content of amaranth grain is almost double that of maize grain.

The mineral content of amaranth obtained in this study compared well with that reported by Teutonico and Dietrich (1985), Pedersen *et al* (1987) and Pond *et al*. (1991). However, phosphorus and magnesium contents reported by these authors were slightly lower. In comparison, maize grain contains a lower mineral content than grain amaranth (Appendix 1). Utilisation of minerals in amaranth might however be hindered by the presence of high level of phytate reported to be 0.32-0.46% by Pedersen *et al* (1987) and 0.52-0.61% by Lorenz and Wright (1984).

3.4.2 Metabolisable energy

Results of energy content of the three grain amaranth species are presented in Table 3. The apparent metabolisable energy (AME) was in the range of 3285-3348 kcal/kg while the true metabolisable energy (TME) was 3832-3903 kcal/kg. *A. hypochondriacus* had a higher metabolisable energy content than the other two species, regardless of its low gross energy. This reflected better utilisation of *A. hypochondriacus* compared to the other two species. The metabolisable energy values from the three species were higher than those reported by Bressani *et al*, (1992) of 2910 and 2790 kcal/kg for light and dark coloured raw amaranth grain respectively. Although *A. caudatus* had the highest gross energy, it had a low AME indicating poor digestibility. This was consistent with its higher crude fibre, tannin and trypsin inhibitor levels (Table 3) all of which could interfere with digestibility. The results of this study showed that grain amaranth had a similar metabolisable energy level to that of maize grain (Appendix 1).

3.4.3 Antinutritional factors

The levels of tannins and trypsin inhibitor of raw grain amaranth are shown in Table 3. The mean tannin content was 0.093%. *A. caudatus* had a higher level of tannins (0.20%) compared to the other species. On the whole, tannin levels were similar to those obtained by Lorenz and Wright (1984) and Teutonico and Dietrich (1985) for *A. cruentus* (0.043-0.13%)

Table 3: Energy, tannin and trypsin inhibitor levels of 3 species of grain amaranth[1]

Species	A. hypochondriacus	A.cruentus	A.caudatus	
Variety	1024	434	1113	Mean
Gross energy (GE),kcal/kg	4582	4554	4627	4588
Apparent metabolisable energy (AME),kcal/kg	3348	3285	3296	3310
True metabolisable energy (TME),kcal/kg	3903	3832	3854	3863
AME, % of GE	73.07	72.13	71.23	72.14
Tannin (Catechin equivalent), %	0.020	0.059	0.20	0.093
Trypsin inhibitor, units/mg	0.55	0.68	0.71	0.65

[1]Air dry basis

and *A. hypochondriacus* (0.054-0.065%). These values were low compared to those of tannins in sorghum and millet which could go beyond 3% (Lorenz and Wright, 1984) and even reach 13.8% (Jacob, 1993).

The mean trypsin inhibitor content was 0.65 units per milligram of grain amaranth. The levels in *A. caudatus* and *A. cruentus* species were higher than in *A. hypochondriacus*. The trypsin inhibitor level in *A. hypochondriacus* was similar to that reported by Keoppe *et al*. (1985 and 1987). The determined trypsin inhibitor content of raw soyabean and soyabean meal in the present study was 2.23 and 0.38 TIU/mg respectively. This showed that grain amaranth had a higher trypsin inhibitor content than soyabean meal, but contained only about one third of the inhibitor level found in raw soyabean. Koeppe *et al*. (1985) reported low levels of trypsin inhibitor in cereal grains, being 0.35 and 0.10 TIU/mg in maize and wheat respectively.

3.4.4 Amino acid profiles

The amino acid composition of the three species of grain amaranth is shown in Table 4. The mean lysine, methionine, cystine and leucine contents were 0.78, 0.32, 0.33 and 0.79% respectively. *A. caudatus* had higher levels of essential amino acids compared to the other species. This was consistent with its higher protein content. *A. cruentus* showed superiority compared to *A. hypochondriacus* species with respect to lysine, methionine and cystine levels. The amino

Table 4: Amino acid composition of 3 species of grain amaranth[1]

Species	A.hypochondriacus			A.cruentus	A.caudatus	
Variety	1023	1024	Jumla	434	1113	Mean
Amino acid, %						
Alanine	0.53	0.50	0.55	0.56	0.60	0.55
Arginine	1.12	1.17	1.12	1.30	1.38	1.22
Aspartic acid	1.08	1.15	1.15	1.22	1.35	1.19
Cystine	0.32	0.30	0.33	0.33	0.38	0.33
Glutamic acid	2.73	2.39	2.87	2.58	2.52	2.62
Glycine	0.95	1.05	1.05	1.12	1.13	1.06
Histidine	0.33	0.37	0.34	0.40	0.43	0.37
Isoleucine	0.48	0.49	0.43	0.53	0.58	0.50
Leucine	0.75	0.77	0.73	0.81	0.89	0.79
Lysine	0.71	0.78	0.75	0.79	0.87	0.78
Methionine	0.31	0.31	0.34	0.32	0.34	0.32
Phenylalanine	0.57	0.52	0.56	0.56	0.61	0.56
Proline	0.61	0.59	0.59	0.66	0.70	0.63
Serine	0.81	0.82	0.89	0.86	0.90	0.86
Threonine	0.49	0.49	0.51	0.52	0.58	0.52
Tyrosine	0.48	0.45	0.44	0.50	0.51	0.48
Valine	0.52	0.55	0.50	0.59	0.64	0.56

[1] Air dry basis

acid profiles obtained generally agree with those of Senft (1980), Pedersen *et al.* (1987) and Pond *et al.* (1991). On average, the essential amino acids profile of grain amaranth is superior to that of maize grain which contains 0.2% lysine, 0.18% methionine and 0.18% cystine (Appendix 1). However, maize grain has a higher level of leucine.

On the whole, the variation in chemical composition of grain amaranth in this study might be attributed to the species and varietal differences. Bressani *et al.*, (1992) reported that variability in chemical composition of grain amaranth appeared to result from interactions between the genetic makeup, environmental factors and cultural practices. These authors compared four selected amaranth lines grown in Mexico and Guatemala. The samples from Mexico contained more protein and less fat than those from Guatemala. Similarly, it is expected that the environmental factors and the cultural practices involved in the growing of amaranth in Kenya are likely to be different from those in other countries.

3.5 Conclusions

a) Raw grain amaranth had a mean of 3310 kcal/kg of apparent metabolisable energy, 15.82% crude protein and rich in lysine (0.78%) and methionine (0.32%).

b) The chemical composition varied with species, *A. hypochondriacus* being better than A. caudatus and *A. cruentus* in metabolisable energy, crude protein and essential amino acids.

c) The three species were generally low in antinutritional factors. However, *A. hypochondriacus* had lower levels than *A. caudatus* and *A. cruentus* species.

d) Based only on the chemical evaluation, grain amaranth can replace maize in poultry diets.

CHAPTER 4

4.0 EXPERIMENT 1: FEEDING VALUE OF THREE SPECIES OF RAW GRAIN AMARANTH IN BROILER CHICKEN DIETS

4.1 Introduction

In production of compounded poultry feeds, the energy source most commonly used is maize and its by products while oil seed cakes serve as the major source of protein. However, there has been increasing competition for maize in developing countries, between human and livestock feeding. This is because maize forms the staple diet for the human population. Production of oil seed crops has been declining in recent years. Lack of self-sufficiency in these feedstuffs has created a research trend into alternative sources of energy and protein for poultry feeds. Maize contains about 8-9% crude protein and a metabolizable energy value of about 3,400 kcal/kg. It has been shown in Chapter 3 that grain amaranth has a comparable level of energy to maize. Its protein content and quality is much higher than that of maize. Grain amaranth has the potential to replace maize in poultry feed.

4.2 Objectives:-

a) To determine the feeding value of three species of raw grain amaranth grown in Kenya.

b) To establish the extent to which raw grain amaranth can replace maize and the protein ingredients in broiler diets.

c) To assess the effect of raw grain amaranth diets on body tissues and carcass composition of broiler chicks.

4.3 Materials and methods

4.3.1 Grain amaranth

The grain used in this experiment was obtained from the same source described in Chapter 3. Results of Chapter 3 showed that the three varieties of *A. hypochondriacus* species were generally similar. In the current experiment, one variety of *A. hypochondriacus* species, namely 1024 was selected based on availability and compared to the two varieties, *A.caudatus* 1113 and *A. cruentus* 434. Maize grain is normally incorporated in broiler chicken starter diets up to a maximum of about 60%. To evaluate grain amaranth as a substitute for maize, graded levels of amaranth were required to replace maize up to 60% of the diet.

4.3.2 Other ingredients

Maize grain was obtained from the National Cereals and Produce Board, Nairobi. The other ingredients (except corn oil and salt) were obtained from Unga Feeds Limited, Nairobi.

4.3.3 Experimental procedure

Day old Shaver 'Starbro' broiler chicks were obtained from a commercial hatchery and fed on a commercial starter diet for the first six days. On the seventh day, four hundred chicks of uniform size were selected comprising equal number of males and females. They were divided into forty groups (20 male, 20 female) of ten chicks each. Each group was weighed and alloted a pen with a floor space of $1m^2$, covered with about 10cm deep wood shavings litter. The pens were electrically heated using infrared bulbs to maintain the right temperature for chicks of about 35°C at day old, declining gradually to about 25°C at four weeks of age. The bulbs were suspended above the chick level and were raised as the chicks matured inorder to reduce the temperature. Every four (2 male, 2 female) groups of chicks were randomly allocated to one of 10 experimental diets (Table 5).

The three species of grain amaranth namely *A. cruentus*, *A. hypochondriacus* and *A. caudatus* were each used at 20, 40 and 60% to make diets 2-10 (Table 5). Diet 1 was a maize-soya bean meal control with no amaranth. As the level of amaranth grain increased in diets 2-10, the amount of maize included was reduced proportionately. Corn oil was added to elevate energy levels of the diets. The level of fishmeal in amaranth diets was lower than in the control owing to the high protein and amino acid content in amaranth. All the diets were formulated to be isocaloric and isonitrogenous and to meet NRC (1984) broiler starter requirements. Feed and water were

Table 5: Composition of broiler starter diets used in experiment 1

Amaranth species	Control	A. cruentus		A. hypochondriacus			A. caudatus		
Amaranth level, %	0	20	40	60	20	40	60	20	40	60
Diet	1	2	3	4	5	6	7	8	9	10
Ingredients, %										
Amaranth	0.00	20.00	40.00	60.00	20.00	40.00	60.00	20.00	40.00	60.00
Maize	54.25	36.80	18.60	1.05	36.55	18.35	0.75	37.05	18.85	1.05
Corn oil	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Soyabean meal	30.00	30.00	30.00	30.00	30.00	30.00	30.00	30.00	30.00	30.00
Fish meal	8.00	5.70	3.20	1.00	6.00	3.50	1.30	5.50	3.00	1.00
Limestone	0.80	1.10	1.80	2.00	1.10	1.80	2.00	1.10	1.80	2.00
Dicalcium phosphate	1.20	0.60	0.60	0.20	0.60	0.60	0.20	0.60	0.60	0.20
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin/mineral premix[1]	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Determined analyses[2]										
Dry matter, %	89.51	89.70	88.89	88.95	89.92	89.90	89.66	88.51	88.84	88.65
Crude protein,%	25.92	24.31	24.86	24.54	24.14	23.80	23.88	25.67	24.07	24.68
Ether extract, %	7.01	7.42	7.94	8.86	7.13	7.29	7.66	7.23	7.52	8.29
Crude fibre, %	3.17	3.82	4.41	5.13	3.75	4.04	5.12	4.24	4.85	5.44
Ash, %	5.85	6.23	6.29	6.54	5.89	5.91	6.08	6.02	6.30	6.88
TME, kcal/kg	3732.00	3696.00	3672.00	3614.00	3724.00	3690.00	3673.00	3681.00	3645.00	3602.00

[1]Vitamin mineral premix provided per kg feed:- vitamin A, 1200 IU; vitamin D_3, 300 IU; vitamin E, 10 IU; vitamin K, 4mg; vitamin B_{12}, 0.008mg; nicotinic acid, 20mg; pantothenic acid, 10mg; riboflavin, 6mg; folic acid, 2mg; biotin, 0.08mg; choline, 0.30g; manganese, 62.5mg; zinc, 62.5mg; iodine, 1.5mg; copper, 6.25mg; selenium, 0.2mg; ethoxyquin, 9g.
[2]Air dry basis

provided *ad libitum*. A completely randomised design was used.

By the end of three weeks of experiment (four weeks of age), there was a high mortality that necessitated pooling together of all the birds within each treatment (4 pens) and redistributing them into 3 groups (pens) per treatment. The very weak and sickly birds were eliminated. The experiment was continued for one more week.

At the end of the experiment (five weeks of age), 1 bird per replicate was weighed and then killed by cervical dislocation. After opening the abdominal cavity, the pancreas was carefully excised and weighed. The meat from the right thigh of the sacrificed bird was carefully removed from the femur, chopped into small pieces and thoroughly minced in a blender. Samples were obtained and analysed for moisture, crude protein and ether extract according to the standard AOAC (1984) procedures. Sections of the pancreas, liver and kidney were obtained from the sacrificed bird. They were immediately put in a labelled bottle containing 10% formalin solution ensuring they were fully immersed. The tissues were then prepared as described below for histopathological examination.

4.3.4 Tissue preparation for histopathological examination

The tissues were fixed in 10% formalin solution as previously described, and then trimmed to a thickness of 2-3mm. Thereafter, they were placed in an automatic tissue processor for the following treatments:- Dehydration using 80% ethyl alcohol for the first 4 hours, 96% alcohol for the next

4 hours and lastly, 100% alcohol for 4½ hours; clearing with xylene for a total of 5 hours; impregnation with molten paraffin wax at 60°C for a total of 6 hours. The tissues were then removed from the processor and embedded into wax blocks using a molten wax dispenser. The individual tissue blocks were separated and fixed onto microtome chunks using a searing spatula, after which they were sectioned to 6m thickness and floated on a water bath at 50°C to flatten out. Each section was then placed on a microscope slide and dried in an oven at 60°C for about 1 hour. The tissues were then quickly dewaxed in xylene and washed in alcohol before rehydrating in water. This was followed by the staining process using hematoxylin and eosin. The tissues were then mounted in Destrene 80, Dibutylphthalate and Xylene (DPX) and the slides left to dry before examining them under a microscope.

4.3.5 Data collection

Proximate analyses and true metabolisable energy of the diets were determined as described in Chapter 3. The birds and the feed were weighed once weekly on pen basis. Weekly weight gain was obtained as the difference between the body weights of two consecutive weeks while weekly feed intake was the difference between two consecutive feed measurements. The feed conversion efficiency (FCE) was calculated as a ratio of feed consumed:weight gain. Mortality rate and post mortem results were recorded.

4.3.6 Statistical data analysis

Data obtained up to 4 and 5 weeks of age was analysed using the analysis of variance procedures of the statistical analysis systems (SAS, 1985). Growth and feed intake data from ten diets up to 4 weeks of age was analysed using a two way (diet x sex) analysis of variance. Means were compared using Tukey's multiple range test. Thereafter, the dietary treatments were analysed as a 3 X 4 (variety x level) factorial. Due to the pooling and redistribution of birds within each treatment in the 5th week of age, the sex factor could not be fitted into the model used to analyse data for that week. A regression analysis of chick body weight against dietary amaranth level was done to assess the effect of progressive replacement of maize with either of the amaranth varieties.

4.4 Results and discussion

4.4.1 Chemical composition of the diets

The chemical composition of diets used in this experiment is presented in Table 5. The amaranth diets had higher crude fibre and fat levels compared to the control. The amount of fibre increased with increasing level of grain amaranth. This was a reflection of the high fibre content in this grain, especially in *A. caudatus*. The higher fat and ash levels of the amaranth diets were consistent with the higher fat and mineral content of grain amaranth in comparison to maize. The calculated essential amino acid composition of the diets is

presented in Table 6, together with the NRC (1984) requirement. All the diets met the essential amino acid requirements but were slightly deficient in methionine.

4.4.2 Broiler chick performance

a) 4 weeks of age

Results of body weight, feed intake and feed efficiency are presented in Table 7. The mean body weights for chicks on the control and amaranth diets were 625 and 352 grams respectively. Increasing dietary grain amaranth resulted in a depression in body weight gain and feed intake. Chicks on diets 1 (control) and 5 (20% *A. hypochondriacus*) had the highest ($P<0.05$) body weight and feed intake while diets 4 and 10 (*A. cruentus* and *A. caudatus* at 60% respectively) gave the lowest. In addition, diets 4 and 10 had significantly ($P<0.05$) poorer feed efficiency than the others. This indicated that the poor body weights from the amaranth diets could be attributed to the low intake and poor metabolism of the diets. The chicks on diets 4 and 10 had a poor feather cover, looked emaciated and suffered a high mortality. Some of the chicks exhibited star-gazing symptoms of thiamin deficiency while others had severe coccidiosis.

The mean mortality rates for chicks on the control, *A. cruentus*, *A. hypochondriacus* and *A. caudatus* dietary treatments were 2.5, 29, 15 and 35% respectively. As the weight gain decreased with increasing level of dietary amaranth, the mortality rate increased. The performance of

Table 6: Amino acid composition of the diets (%)

Amaranth species	Control	A.cruentus			A. hypochondriacus			A. caudatus			Requirement[1]
Amaranth level,%	0	20	40	60	20	40	60	20	40	60	
Diet	1	2	3	4	5	6	7	8	9	10	
Amino acid											
Arginine	1.54	1.59	1.63	1.72	1.59	1.66	1.72	1.64	1.73	1.88	1.44
Histidine	0.55	0.55	0.55	0.57	0.55	0.56	0.56	0.57	0.58	0.62	0.35
Isoleucine	1.26	1.20	1.12	1.09	1.21	1.17	1.12	1.22	1.17	1.18	0.80
Leucine	2.02	1.87	1.69	1.57	1.87	1.73	1.59	1.89	1.74	1.67	1.35
Lysine	1.38	1.39	1.38	1.44	1.38	1.40	1.41	1.40	1.42	1.51	1.20
Methionine	0.44	0.44	0.43	0.44	0.43	0.43	0.43	0.43	0.42	0.44	0.50
Phenylalanine	1.18	1.15	1.10	1.09	1.15	1.12	1.09	1.15	1.11	1.12	0.72
Threonine	0.97	0.94	0.91	0.91	0.94	0.92	0.89	0.95	0.93	0.95	0.80
Tryptophan	0.30	0.31	0.31	0.32	0.31	0.32	0.32	0.30	0.31	0.32	0.23
Valine	1.18	1.14	1.08	1.07	1.14	1.11	1.08	1.16	1.13	1.15	0.82

[1]NRC (1984)

Table 7: Broiler performance at 1-4 weeks of age

Species	Control	A. cruentus			A. hypochondriacus			A. caudatus		
Amaranth,%	0	20	40	60	20	40	60	20	40	60
Diet	1	2	3	4	5	6	7	8	9	10
Response										
Body weight, (g/bird)	625[a]	503[bc]	273[e]	140[f]	563[ab]	458[c]	368[d]	433[cd]	272[e]	158[f]
Feed intake, (g/bird)	1125[a]	908[b]	593[de]	410[e]	1040[ab]	1018[ab]	870[bc]	833[bc]	660[cd]	418[e]
Feed conversion Efficiency (Feed:gain)	2.14[b]	2.25[b]	3.43[b]	10.25[a]	2.25[b]	2.84[b]	3.25[b]	2.50[b]	3.84[b]	7.21[a]

[ab]Means with the same superscript within the row are not different (P>0.05).

chicks on the high amaranth level treatments started to improve by the 4th and 5th weeks of age.

Table 8 shows the effect of amaranth species and inclusion level on chick performance. Of the three species, *A. hypochodriacus* gave the highest ($P<0.05$) body weight and feed intake and a better ($P<0.05$) feed efficiency. Each successive dietary increment in amaranth level caused a reduction ($P<0.05$) in chick body weight and feed intake. Male and female chicks had similar ($P>0.05$) body weight, feed intake and feed efficiency. The effect of progressive dietary increase of amaranth on chick body weight is shown by the regression curves in Figure 1. Replacement of dietary maize with amaranth caused a linear decline in chick body weight, the effect being more gradual with *A. hypochondriacus* than with the other two species.

The results of this study are in agreement with findings by various authors. The presence of antinutritional factors in the amaranth diets could have contributed to the depressed feed intake and the concomitant poor chick growth. These factors might have caused an objectionable taste thus reducing palatability of the feed. Cheeke and Bronson (1980) found that 65% amaranth diets fed to rats were very unpalatable and caused the rats to exhibit symptoms typical of semi-starvation. These researchers concluded that raw amaranthus seed contained a factor(s) which resulted in low palatability. They further suggested that the likely candidates were saponins and phenolic compounds both of which were astringent

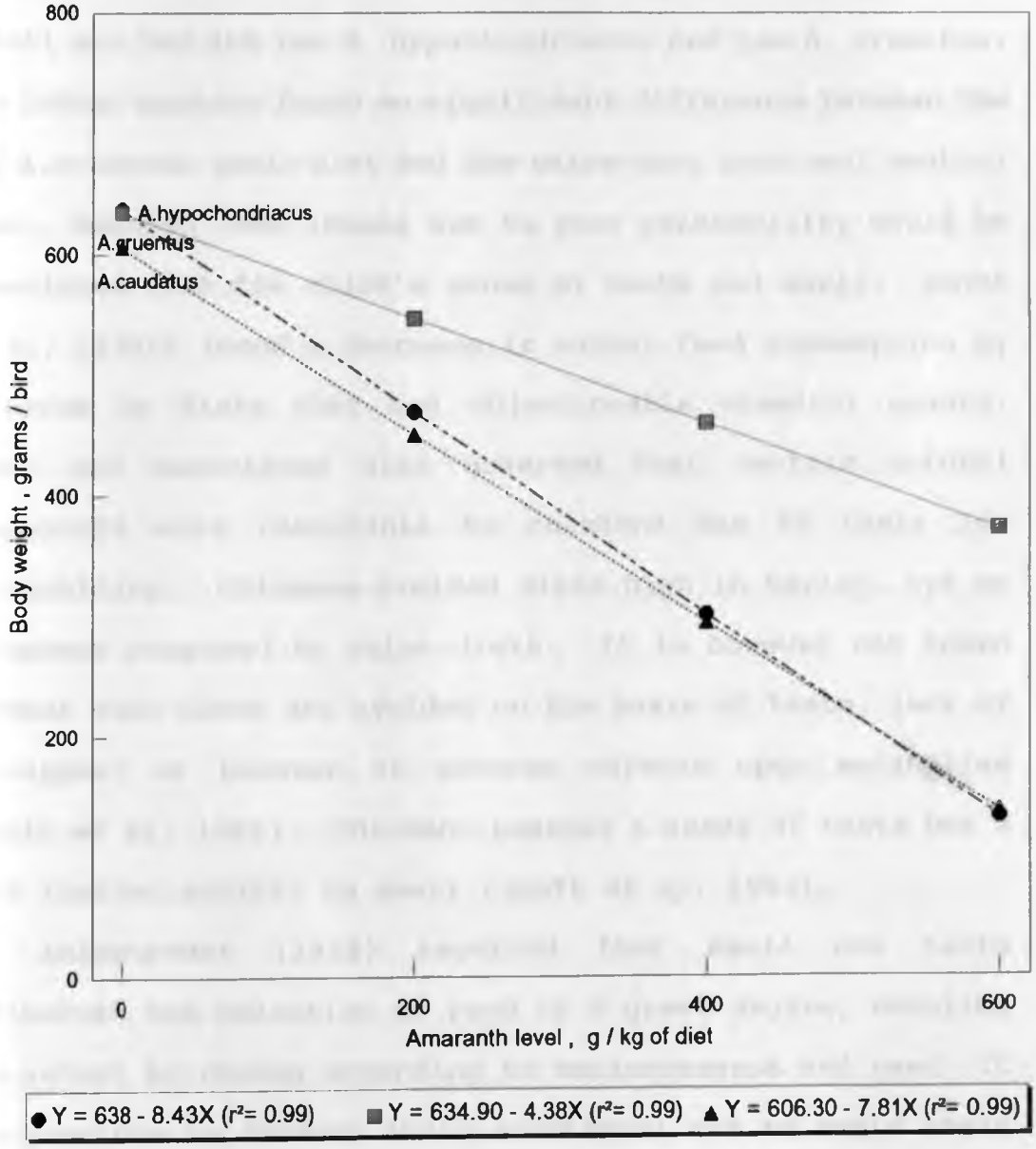
Table 8: Effect of sex, amaranth species and inclusion level on broiler performance at 1-4 weeks of age

		Body weight (g/bird)	Feed intake (g/bird)	Feed efficiency (Feed:gain)
Sex	Males	388[a]	795[a]	2.35[a]
	Females	370[a]	780[a]	2.43[a]
Species	A. cruentus	305[b]	646[b]	5.31[a]
	A. hypochodriacus	463[a]	976[a]	2.78[b]
	A. caudatus	288[b]	637[b]	4.52[a]
Level, %	0	625[a]	1125[a]	2.13[b]
	20	499[b]	927[b]	2.33[b]
	40	334[c]	757[c]	3.37[b]
	60	222[d]	566[d]	6.90[a]

[ab]Means with the same superscript for each factor within a column are not different ($p>0.05$).

Fig.1. Effect of amaranth level and species

On chick body weight at 4 weeks of age



in taste. Pond *et al.* (1991) reported that *A. hypochondriacus* and *A. cruentus* grain had growth inhibiting factors after feeding rats on raw and heat treated grain. Depressed feed intake and growth in chickens was reported by Laovoravit *et al.* (1986) who fed 30% raw *A. cruentus* and Waldroup *et al.* (1985) who fed 40% raw *A. hypochondriacus* and raw *A. cruentus*. The latter authors found no significant difference between the 20% A.cruentus grain diet and the maize-soya bean meal control diet. Reduced feed intake due to poor palatability would be associated with the chick's sense of taste and smell. Scott *et al.* (1982) found a decrease in normal feed consumption by chickens in diets that had objectionable chemical agents. Scott and associates also observed that certain natural feedstuffs were unsuitable to chickens due to their low palatability. Chickens avoided diets high in barley, rye or buckwheat compared to maize diets. It is however not known whether such diets are avoided on the basis of taste, lack of eye-appeal or because of adverse effects upon metabolism (Scott *et al*, 1982). Chickens possess a sense of taste but a very limited ability to smell (Scott *et al*, 1982).

Leibetseder (1978) reported that smell and taste influenced the selection of food to a great degree, enabling the animal to choose according to wholesomeness and need. It was possible to discern toxic substances and to avoid their intake. Pleasant or unpleasant smell and taste influenced the hunger and satiety centres in the hypothalamus to either increase or decrease feed intake as a regulatory mechanism

(Leibetseder, 1980).

In addition to reduced palatability, the antinutritional factors in amaranth diets might have inhibited the availability of nutrients for metabolism. Tannins and trypsin inhibitor are known to interfere with protein digestibility and hence reduce availability of amino acids. Herkelman *et al.* (1993) found depressed performance and nitrogen retention when chicks were fed either low trypsin-inhibitor or conventional soyabeans in the raw form. If amino acid availability is hampered, an imbalance could be created in the animal resulting in a subsequent depression in feed intake. Harper *et al* (1970) reported that the depressed feed intake response resulting from amino acid imbalance was a homeostatic mechanism which prevented an accumulation of amino acids that were not useful to the animal for tissue protein synthesis. The diets used in the present study were formulated to have adequate levels of essential amino acids (Table 6). Theoretically, the chicks should therefore not have suffered inadequacy or imbalance of amino acids.

In this study, *A. hypochondriacus* gave the best ($P<0.05$) performance. This agrees with results by Cheeke and Bronson (1980) but is contrary to findings of Waldroup *et al.* (1985) who found a more severe reduction in performance with chicks fed on this species. In the results of Chapter 3 (Table 4), it was noted that *A. hypochondriacus* had lower levels of tannins and trypsin inhibitor than the other two species. A similar trend was reported for lectin content by Koeppe and

Rupnow (1988). This might explain the better performance of this species. It could also explain the lower mortality since the feed intake and weight gain remained higher with this species than with the other two. Lectins are known to impair the immune system leading to greater sensitivity to bacterial infections (Cheeke and Shull, 1985). The birds on *A. hypochondriacus* treatments were much healthier than those on the other two species and appeared almost similar to those on the control diet.

The lack of differences in performance between males and females up to the 4th week of age was contrary to work reported by Scott *et al.* (1982) showing faster growth rates for males than for females. Sex differences in this study might have appeared if the experiment was extended for a longer period.

b) 5 weeks of age.

Results of feed intake, body weight and feed efficiency at 5 weeks of age are presented in Table 9. The mean body weights for chicks on the control and amaranth diets were 928 and 665 grams respectively. During the fifth week of age, chick weight gain markedly improved by margins similar to that of the control diet. The improvement was most striking in the 40 and 60% levels of *A. cruentus* and *A. caudatus* where performance was initially very poor. Feed intake equally improved for both species but a considerable drop was observed as the amaranth level increased from 20 to 60%. The high weight gain resulting from lower feed intake for the high

Table 9: Broiler performance at 4-5 weeks of age

Species	Control	A. cruentus			A. hypochondriacus			A. caudatus		
% Amaranth	0	20	40	60	20	40	60	20	40	60
Diet	1	2	3	4	5	6	7	8	9	10
Response										
Body weight, (g/bird)	928[a]	839[ab]	602[cde]	467[f]	867[ab]	750[bcd]	643[cde]	770[abc]	590[def]	463[f]
Feed intake, (g/bird)	565[a]	563[a]	490[a]	461[a]	573[a]	531[a]	497[a]	570[a]	503[a]	465[a]
Weight gain, (g/bird)	303[a]	336[a]	329[a]	327[a]	304[a]	292[a]	275[a]	337[a]	318[a]	305[a]
Feed efficiency (Feed: gain)	1.86[a]	1.68[ab]	1.49[b]	1.41[b]	1.88[a]	1.82[a]	1.81[a]	1.69[ab]	1.58[b]	1.52[b]
Pancreas weight (% body weight),5 weeks of age	0.32[c]	0.36[bc]	0.44[abc]	0.54[ab]	0.34[c]	0.39[bc]	0.44[abc]	0.32[c]	0.54[ab]	0.58[a]

[ab]Means with the same superscript within a row are not different (P>0.05) .

amaranth level diets meant better feed utilisation as reflected in the feed efficiency ratios.

Table 10 shows the similarity in chick weight gain between the different species and between the four inclusion levels of amaranth in the fifth week of age. While the three species gave similar ($P>0.05$) feed intake, there was reduction in this parameter as the dietary amaranth level increased beyond 20%. During this period, *A. cruentus* and *A. caudatus* gave better ($P<0.05$) feed efficiency than *A. hypochondriacus*. This was contrary to results of feed efficiency obtained at four weeks of age.

Figure 2 shows the linear regression curves of chick body weight against increasing dietary levels of grain amaranth at five weeks of age. By the end of the fifth week of age, the chicks appeared to have overcome the negative factors that caused the early depression in feed intake. Compensatory growth appears to have occurred following the period of underfeeding. This was in agreement with findings by Scott *et al.* (1982) that healthy chickens given no choice will gradually increase consumption of many feeds initially avoided, ultimately resulting in normal performance. Retarded growth caused by feed restriction was followed by more rapid growth during subsequent liberal feeding (Griffith *et al*, 1977; Maynard *et al*, 1979). Such animals were capable of recovering and eventually reaching the same size as the continually well fed animals (Maynard *et al*, 1979).

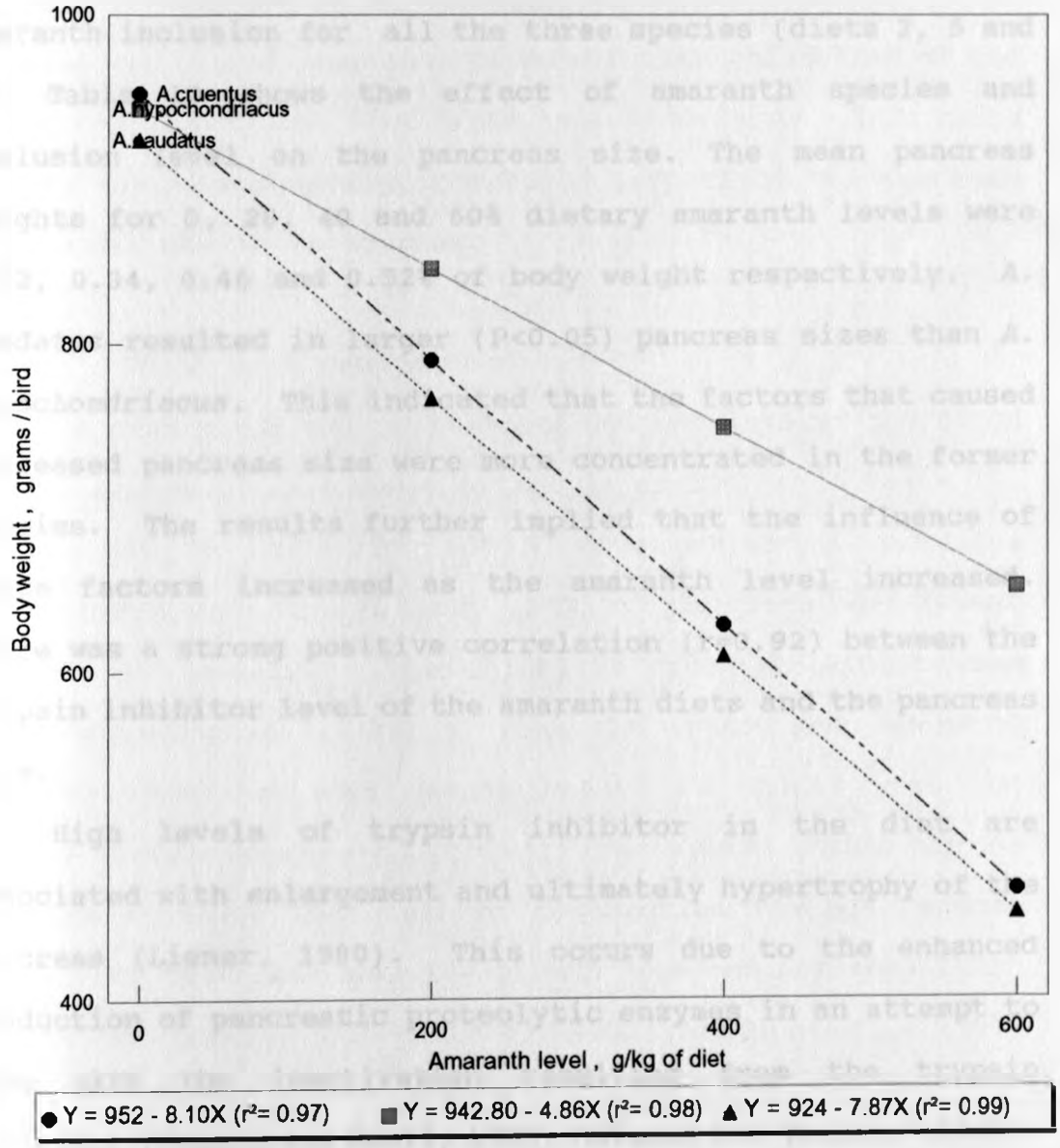
Table 10: Effect of amaranth species and inclusion level on broiler performance at 4-5 weeks of age

		Weight gain (g/bird)	Body weight (g/bird)	Feed Intake (g/bird)	Feed efficiency (Feed:gain)	Pancreas weight (% body weight)
Species	A. cruentus	331[a]	636[b]	505[a]	1.53[b]	0.45[ab]
	A. hypochondriacus	290[a]	753[a]	534[a]	1.84[a]	0.39[b]
	A. caudatus	320[a]	608[b]	513[a]	1.60[b]	0.48[a]
Level, %	0	303[a]	928[a]	565[a]	1.86[a]	0.32[b]
	20	326[a]	825[a]	569[a]	1.75[a]	0.34[b]
	40	313[a]	647[b]	508[ab]	1.63[a]	0.46[a]
	60	302[a]	524[c]	474[b]	1.58[a]	0.52[a]

[ab]Means with the same superscript for each factor within a column are not different (P>0.05).

Fig.2. Effect of amaranth level and species

On chick body weight at 5 weeks of age



4.4.3 Pancreas Weight

The pancreas weight as a percentage of the body weight at 5 weeks of age is presented in Table 9. The higher the level of amaranth in the diet, the higher the weight of the pancreas. The control diet was similar to the 20% amaranth inclusion for all the three species (diets 2, 5 and 8). Table 10 shows the effect of amaranth species and inclusion level on the pancreas size. The mean pancreas weights for 0, 20, 40 and 60% dietary amaranth levels were 0.32, 0.34, 0.46 and 0.52% of body weight respectively. *A. caudatus* resulted in larger ($P<0.05$) pancreas sizes than *A. hypochondriacus*. This indicated that the factors that caused increased pancreas size were more concentrated in the former species. The results further implied that the influence of these factors increased as the amaranth level increased. There was a strong positive correlation ($r=0.92$) between the trypsin inhibitor level of the amaranth diets and the pancreas size.

High levels of trypsin inhibitor in the diet are associated with enlargement and ultimately hypertrophy of the pancreas (Liener, 1980). This occurs due to the enhanced production of pancreatic proteolytic enzymes in an attempt to cope with the inactivation resulting from the trypsin inhibitor (Cheeke and Shull, 1985; Huisman and Tolman, 1992).

Results of the current study agree with those of Chubb (1982) who found that diets containing moderate levels of trypsin inhibitor depresssed growth and produced a slightly

enlarged pancreas (0.44% of body weight). However, Liener and Kakade (1980) gave the normal size of chick pancreas as 0.4-0.6% of body weight. All the pancreas weights obtained in the present study were within this range. It was shown in table 4 (Chapter 3) that the trypsin inhibitor content of *A. caudatus* was higher than that of *A. cruentus* while that of the latter was higher than that of *A. hypochondriacus*. This could explain the corresponding increasing pattern of the pancreas weights for the three species.

4.4.4 Thigh meat composition

The mean moisture, protein and fat contents of the thigh meat of chicks at five weeks of age is presented in Table 11. Diets 4 and 10 containing 60% *A. cruentus* and *A. caudatus* respectively gave the highest ($P<0.05$) moisture and lowest ($P<0.05$) fat content. The chicks in these treatments had poorer weight gains (Table 9) than the others and deposited less fat. The low body weight meant the chicks were not getting enough energy and protein for growth. This agrees with the findings that caloric restriction through reduced feed intake decreased both the growth rate and fat deposition in broilers (Mabray and Waldroup, 1981; Arafa *et al.*, 1983; Lipstein, 1989).

By the fifth week of age, birds in these treatments might not have attained a status of large excesses of energy in the body which could have been deposited as fat. The low fat and high moisture levels of these chicks illustrates the

Table 11: Thigh meat composition (%) of broilers at 5 weeks of age

Species	Control	A. cruentus			A. hypochondriacus			A. caudatus		
Amaranth,%	0	20	40	60	20	40	60	20	40	60
Diet	1	2	3	4	5	6	7	8	9	10
Moisture	73.42^{b}	73.51^{b}	74.01^{b}	75.70^{a}	73.32^{b}	73.18^{b}	73.74^{b}	73.43^{b}	74.72^{b}	76.43^{a}
Protein	17.43^{b}	17.29^{b}	18.84^{a}	19.26^{a}	18.08^{ab}	18.48^{ab}	18.63^{ab}	19.02^{a}	19.03^{a}	19.41^{a}
Fat	7.85^{a}	7.37^{a}	5.99^{a}	3.41^{c}	7.29^{a}	6.47^{ab}	6.35^{ab}	5.70^{b}	5.49^{b}	3.13^{c}

^{ab}Means with the same superscript within a row are not different (P>0.05)

Correlation coefficients:

Fat vs moisture; r = -0.92 ; P = 0.0002*
Fat vs protein; r = -0.88 ; P = 0.0010*

hypothesis that as the body fat level increases, the moisture content reduces. Lipstein, (1989) reported that fat is deposited into tissue at the expense of water. The dietary treatments that had faster rate of chick growth such as the control diet and 20% *A. hypochondriacus* had higher fat levels and less moisture in the thigh meat. McDonald *et al.* (1981) reported that gains made by faster growing animals contain greater concentrations of fat and energy and lesser concentration of water, protein and ash. The highly negative correlation coefficients for moisture versus fat and protein versus fat (Table 11) illustrate this relationship.

4.4.5 Histopathology

Results of the microscopic examination of the various tissues did not show significant differences between the dietary treatments. In the pancreas, the changes found in some birds included acinar dissociation, patchy areas of necrosis and leucocytic infiltration. The severity was variable. The secretory activity also varied, even within the same treatment group. In the liver, the hepatocytes of a few birds showed degenerative and necrotic changes. There was some extent of leucocytic infiltration. Similar observations were noted in the kidney where a few birds showed degenerative changes in the tubular epithelium and some interstitial leucocytic infiltration.

There were no specific patterns consistent with the different dietary treatments. The changes were generally

moderate although their severity varied between individual birds and dietary treatments. The lack of significant differences between the control and the amaranth diets indicated that the changes were not associated with the feeding of amaranth.

4.5 Conclusions

a) Raw *A. hypochondriacus* grain can replace maize grain in broiler chicken diets but only at 20% level.

b) As the amaranth level increased, feed intake, body weight and feed efficiency decreased while the pancreas weight increased.

c) Relatively higher liveweight gains were obtained from *A. hypochondriacus* based diets than those based on *A. cruentus* or *A. caudatus*.

d) Chicks on high amaranth level diets had low fat, high moisture carcasses concomitant with their poor body weight.

e) Histopathology showed some changes in the liver, pancreas and kidney of a few birds which could not be attributed to the feeding of grain amaranth.

CHAPTER 5

5.0 EXPERIMENT 2: FEEDING VALUE OF THERMAL PROCESSED GRAIN AMARANTH

5.1 Introduction

In Experiment 1, chick performance on raw grain amaranth diets was poor compared to the maize control diet. The performance declined with increasing dietary levels of amaranth despite the fact that grain amaranth has high levels of energy and protein, with a good amino acid profile. Clearly, this shows that there may be factors which limit the use of grain amaranth. Thermal treatment is known to destroy various antinutritional factors. Grain amaranth used in this experiment was therefore subjected to thermal treatment.

5.2 Objectives

a) To determine the effect of thermal treatment on chemical composition of grain amaranth,

b) To determine the effect of the treated grain on broiler chicken performance, and

c) To assess the effect of heat treated grain amaranth diets on body tissues and carcass composition.

5.3 Materials and methods

5.3.1 Grain amaranth

Out of the 3 grain species used in experiment 1, *A. hypochondriacus* (1024) was selected for this experiment due to its better performance. Amongst the other two species, *A. cruentus*, variety 434 was selected due to its high availability.

5.3.2 Heat processing (extrusion) of the grain

The above processing was carried out by Supa Snacks Limited, Nairobi. A cereal puffing machine with a thermo controller was used. The machine was similar to the collet extruders described by Lusas and Riaz (1994) for making puffed snacks from corn grit (see literature review). The two grain species were processed separately. The procedure was as follows:- the amaranth grain was first moistened with water, then passed through the cereal puffing machine set at 150°C. The grain was quickly processed, leaving the machine in form of long, puffed up strands which broke up into lightweight pellets. This low-density product was collected in large trays, cooled and then ground in a hammer mill to a suitable (medium particle) feed size. It was then incorporated into the experimental diets.

5.3.3 Chemical analyses

Samples of the heat processed grain amaranth were subjected to the same chemical analyses described for the raw

grain in Chapter 3. These were:- proximate and mineral analyses, energy determination, analyses for tannins, trypsin inhibitor and amino acids.

5.3.4 Experimental procedure

Two hundred and eighty day old 'Arbor acres' female chicks were obtained from a commercial hatchery. On the second day, they were divided into 28 groups of 10 chicks each, weighed and placed in 28 pens similar to those used in Experiment 1. Each group was assigned one of seven experimental broiler starter diets (Table 12). Each of the seven dietary treatments was replicated four times in a completely randomized design. Diet 1 was a maize-soya bean meal control with no amaranth. Diets 2-7 were made of heat processed *A. hypochondriacus* and *A. cruentus* grain included at 20, 40 and 60%. Fish meal was incorporated at increasing levels as the amaranth content decreased inorder to meet the protein requirement. This was necessary since the heat-treated amaranth contained slightly less protein compared to the raw one. The chicks were on experimental diets for four weeks. Feed and water were provided *ad libitum*. The brooding management was as described in Experiment 1.

At the end of the experimental period, one bird per replicate was sacrificed and various tissues obtained and treated as described in Experiment 1.

Table 12: Composition of broiler starter diets used in Experiment 2

Amaranth species	Control	A. hypochondriacus			A. cruentus		
Amaranth level, %	0	20	40	60	20	40	60
Diet	1	2	3	4	5	6	7
Ingredients %							
Maize	54.25	36.40	18.15	0.00	36.65	18.15	0.63
Amaranth	0.00	20.00	40.00	60.00	20.00	40.00	60.00
Corn oil	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Soyabean meal	30.00	30.00	30.00	30.00	30.00	30.00	30.00
Fish meal	8.00	6.00	4.20	2.30	5.70	4.20	1.50
Limestone	0.80	1.05	1.50	1.95	1.05	1.50	2.00
Dicalcium phosphate	1.20	0.80	0.40	0.00	0.85	0.40	0.12
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin mineral premix[1]	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Determined analyses (Air dry basis)							
Dry matter, %	90.16	90.82	91.42	91.53	90.49	90.83	91.62
Crude protein, %	23.69	24.62	23.86	23.54	24.09	24.80	23.88
Ether extract, %	6.87	6.92	7.11	7.56	7.33	8.01	8.32
Crude fibre, %	3.34	3.62	4.07	3.83	3.86	4.35	4.86
Ash, %	5.68	5.43	5.57	5.81	5.59	5.85	5.89
TME, kcal/kg	3776.00	3749.00	3801.00	3826.00	3725.00	3770.00	3815.00
Calculated analysis							
Lysine, %	1.34	1.37	1.37	1.36	1.37	1.40	1.38
Methionine, %	0.44	0.43	0.42	0.40	0.43	0.43	0.42
Cystine, %	0.37	0.38	0.39	0.40	0.39	0.41	0.41

[1]Vitamin mineral premix provided per kg feed:- Vitamin a, 1200 IU; Vitamin D_3, 300 IU; Vitamin E, 10IU; Vitamin K, 4mg; Vitamin B_{12}, 0.008mg; Nicotinic acid, 20mg; Pantothenic acid, 10mg; riboflavin, 6mg; folic acid, 2mg; biotin, 0.08mg; choline, 0.30g; manganese, 62.5mg; zinc, 62.5mg; iodine, 1.5mg; Copper, 6.25mg; Selenium, 0.2mg; ethoxyquin, 9g.

5.3.5 Data Collection

This was carried out as described in Experiment 1.

5.3.6 Statistical data analysis

Analysis of variance procedures of the Statistical Analysis Systems (SAS, 1985) were used. After the initial comparison of the seven diets, the various treatments were analysed as a 2 x 4 (variety x level) factorial. Means were compared using Tukey's multiple range test. A regression analysis of chick body weight against dietary amaranth level was carried out.

5.4 Results and discussion

5.4.1 Chemical analyses of processed grain amaranth

Results of proximate, mineral, energy, tannin and trypsin inhibitor determinations are presented in Table 13. *A. cruentus* species had a superior proximate and mineral composition compared to *A. hypochondriacus*. This finding was consistent with that of similar raw grain species in Chapter 3. However, the proximate fractions of both species were lower compared to those of the corresponding raw grain species.

The mean apparent metabolisable energy for processed grain amaranth was 3401 kcal/kg. *A. hypochondriacus* showed a higher energy content than *A. cruentus*. The tannin and trypsin inhibitor levels were considerably lower than those of the raw grain of similar species in Chapter 3. There was no

Table 13: Chemical composition of 2 species of heat processed grain amaranth[1]

Species variety	A. hypochondriacus 1024	A. cruentus 434	Mean
Dry matter, %	91.50	92.07	91.79
Crude Protein %	13.97	14.58	14.28
Ether extract, %	5.98	6.85	6.42
Crude fibre, %	4.67	4.86	4.77
Ash, %	2.72	3.01	2.87
Calcium, %	0.19	0.16	0.18
Magnesium, %	0.50	0.51	0.51
Phosphorus, %	0.53	0.60	0.57
Iron, %	0.14	0.15	0.15
Potassium, %	0.34	0.36	0.35
Sodium, ppm	61.00	65.00	63.00
Manganese, ppm	26.00	35.00	30.50
Copper, ppm	3.00	3.00	3.00
Zinc, ppm	29.00	32.00	30.50
Gross energy, kcal/kg	4446.00	4389.00	4417.50
Apparent metabolisable energy, kcal/kg	3470.00	3332.00	3401.00
True metabolisable energy, kcal/kg	4033.00	3895.00	3964.00
Tannin (Catechin equivalent, %	0.014	0.026	0.02
Trypsin inhibitor, units/mg	0.20	0.22	0.21

[1] Air dry basis

difference in these levels between the two processed grain species, contrary to the results of the raw grain.

The improvement in metabolisable energy after heat processing reflected better digestibility and utilisation of the processed grain compared to the raw one. The metabolisable energy values of the processed grain amaranth in this study were higher than those reported by Laovaravit *et al.*, (1986) for autoclaved *A. cruentus*. However, they were similar to those reported by Tillman and Waldroup (1988a) and by Bressani *et al.* (1992) for extruded *A. cruentus* grain. Peskier (1994) explained that significant improvements in digestibility and metabolisable energy in thermal expanded feedstuffs occured due to destruction of antinutritional factors, gelatinisation of starch which improves digestibility, release of natural fat from the feedstuff making it more accessible to digestive enzymes and finally, increasing solubility of fibre which enhances its digestibility. The reduction of antinutritional factors in grain amaranth by heat processing was in agreement with findings reported by Koeppe *et al* (1987) and Pedersen *et al* (1987).

Results of the amino acid composition are presented in Table 14. The amino acid profiles showed reduction in the levels of certain amino acids compared to those of similar raw grain species in Chapter 3, Table 4. The lysine content in heat processed *A. hypochondriacus* (1024) grain was 0.63% while in the raw grain it was 0.78%. However, *A. cruentus* remained

Table 14: Amino acid composition of 2 species of processed grain amaranth[1]

Species	A.hypochondriacus	A.cruentus	
Variety	1024	434	Mean
Amino acid, %			
alanine	0.46	0.50	0.48
Arginine	0.94	1.05	1.00
Aspartic acid	0.97	1.07	1.02
Cystine	0.30	0.33	0.32
Glutamic acid	2.12	2.34	2.23
Glycine	0.87	0.96	0.92
Histidine	0.28	0.32	0.30
Isoleucine	0.37	0.43	0.40
Leucine	0.64	0.71	0.68
Lysine	0.63	0.72	0.68
Methionine	0.29	0.32	0.31
Phenylalanine	0.49	0.51	0.50
Proline	0.44	0.47	0.46
Serine	0.77	0.82	0.80
Threonine	0.43	0.49	0.46
Tyrosine	0.41	0.40	0.41
Valine	0.41	0.47	0.44

[1]Air dry basis

superior to *A. hypochondriacus* in conformity with results of the raw grain. Heat processing could have destroyed some of the amino acids and hence reduced the protein content of the processed grain. Maillard reaction might have occurred involving carbohydrate - amino acid reactions, especially in the case of lysine, arginine and histidine (Scott *et al* , 1982). Damage could also occur due to interactions between functional groups within the protein, affecting amino acids such as lysine, glutamic acid, aspartic acid and threonine (Erbersdobler, 1976). Betschart *et al* (1981) found a 15% decrease in lysine content in popped *A. cruentus* seeds and lysine became the second limiting amino acid in rat feeding trials.

5.4.2 Chemical composition of diets

The chemical composition of diets used in this experiment is presented in Table 12. The crude protein content of the diets was in the range of 23.54-24.80% which fulfilled the NRC (1984) requirement.

The true metabolisable energy values were in the range of 3725-3826 kcal/kg. The ether extract and crude fibre levels of the amaranth based diets increased with higher levels of amaranth inclusion. However, the crude fibre levels were generally lower than in Experiment 1. The two species used had less fibre content than *A. caudatus* which was omitted in this experiment. The heat treatment also appeared to have lowered the crude fibre level by small margins.

5.4.3 Broiler chick performance

Results of body weight, feed intake, feed conversion efficiency and pancreas weight at four weeks of age are presented in Table 15. For the amaranth diets, the mean body weight per bird was 743 grams which was higher than that obtained in Experiment 1 at the same age. This weight exceeded the expected mean of 0.66 kg per bird reported by Scott *et al.* (1982). The relatively high body weights attained in this experiment implied that thermal treatment of grain amaranth resulted in a more nutritious feed. Chicks fed diet 1 (control) exhibited similar ($P>0.05$) body weight to that of diets 2 and 3 (20 and 40% *A. hypochondriacus* respectively) and diets 5 and 6 (20 and 40% *A. cruentus* respectively). All the diets gave similar ($P>0.05$) feed intake except diet 7 containing 60% *A. cruentus* grain. No differences ($P>0.05$) occurred in pancreas weight between the different dietary treatments.

When chick performance from the two amaranth species was compared (Table 16) there was similar ($P>0.05$) body weight and feed efficiency. However, chicks on *A. hypochondriacus* diets consumed more ($P<0.05$) feed than those on *A. cruentus*. A comparison by level of dietary amaranth (Table 16) showed similar ($P>0.05$) chick performance at 0, 20 and 40%. High level of dietary amaranth (60%) resulted in lower body weight ($P<0.05$) and lower feed intake ($P<0.05$) than 20% level. However, the feed intake and feed efficiency at 60% amaranth level were not different ($P>0.05$) from those at 40% level.

Table 15: Effect of heat processed grain amaranth on broiler performance at 4 weeks of age

Amaranth species	Control	A. hypochondriacus			A. cruentus		
Amaranth level,%	0	20	40	60	20	40	60
Diets	1	2	3	4	5	6	7
Response							
Body weight, g/bird	792[a]	787[a]	760[ab]	725[ab]	775[a]	732[ab]	677[b]
Feed intake, g/bird	1515[a]	1500[ab]	1508[a]	1475[ab]	1462[ab]	1433[ab]	1372[b]
Feed efficiency (feed:gain)	2.05[b]	2.04[b]	2.17[a]	2.19[a]	2.06[b]	2.11[ab]	2.18[a]
Pancreas weight (% body weight)	0.26[a]	0.29[a]	0.28[a]	0.26[a]	0.26[a]	0.27[a]	0.30[a]

[ab]Means with the same superscript within a row are not significantly different (P>0.05)

Table 16: Effect of amaranth species and inclusion level on broiler performance at 4 weeks of age

	Body weight, (g/bird)	Feed intake, (g/bird)	Feed:efficiency (feed:gain)	Pancreas weight, (% of body weight)
Species				
A. hypochondriacus	742[a]	1494[a]	2.19[a]	0.28[a]
A. cruentus	732[a]	1423[b]	2.10[a]	0.28[a]
Level, %				
0	792[a]	1515[a]	2.05[b]	0.26[a]
20	781[a]	1481[a]	2.05[b]	0.27[a]
40	746[ab]	1470[a]	2.14[ab]	0.28[a]
60	679[b]	1424[b]	2.27[a]	0.28[a]

[ab]Means with the same superscript for each factor within the column are not different (P>0.05)

Figure 3 shows the linear regression curves of the chick body weight against increasing levels of dietary amaranth at four weeks of age. The slope for *A. cruentus* species was almost similar to that of *A. hypochondriacus*. This implied that the chick performance on processed amaranth diets of the two species was more similar than that of the same raw species in Experiment 1.

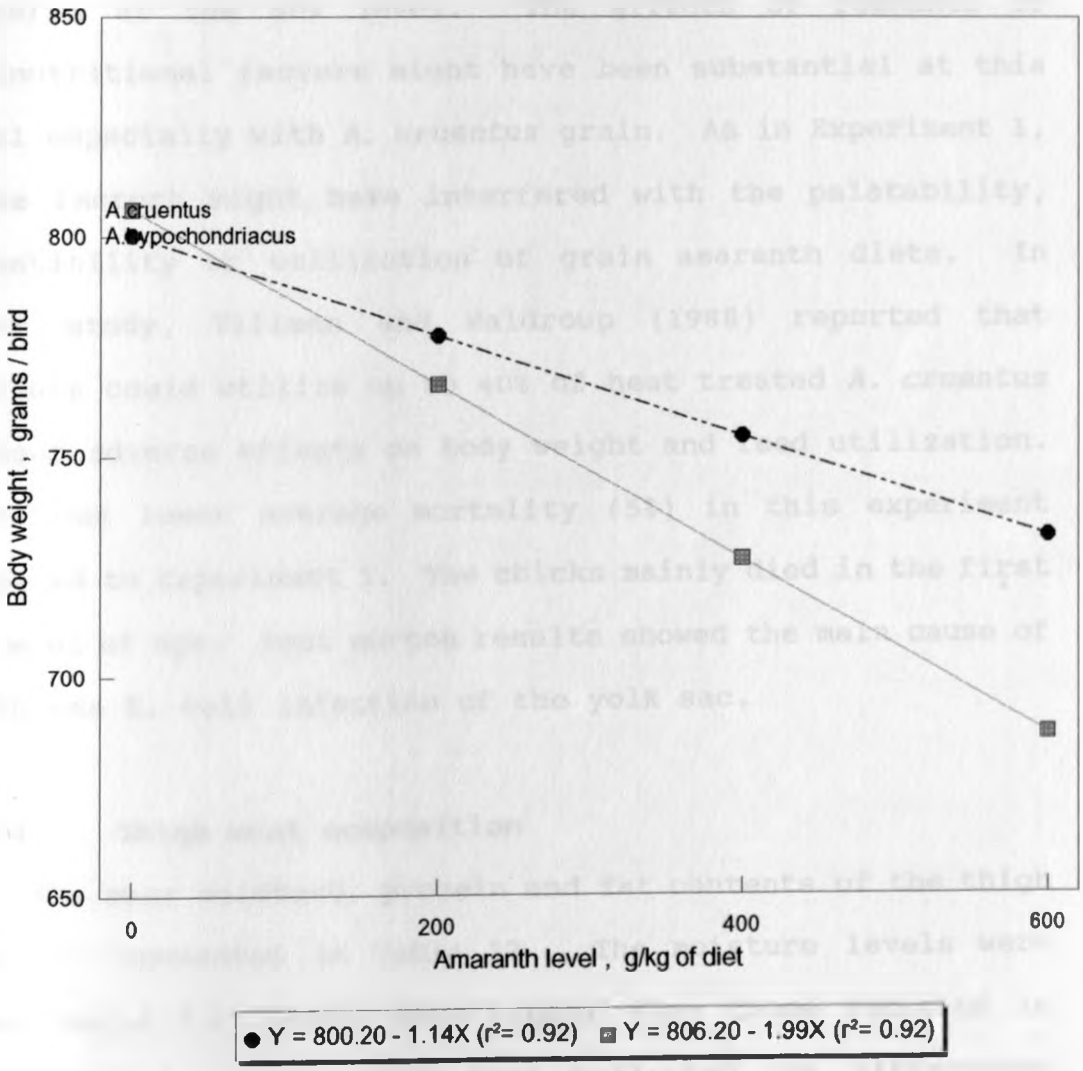
The above results showed marked improvement in chick performance when heat treated amaranth was used. Inclusion of this grain upto 40% had no significant adverse effects on performance (Table 16). However, there was generally a gentle decline in chick body weight as the level of dietary amaranth increased from 0 to 60% (Figure 3). The improvement in weight gain was attributed to the striking increase in feed intake.

Heat processing improved the palatability of the grain thus increasing its acceptability to the chicks. In addition, digestibility and utilisation of the processed grain might have improved as explained by Peskier (1994). At 60% level however, *A. cruentus* was not acceptable enough to give a feed intake equal to that of the other diets.

The heat treatment destroyed some of the anti-nutritional factors in grain amaranth which caused low palatability. Evidence of this is shown in Table 13 where the levels of trypsin inhibitor and tannins were much lower than those found in the raw grain in Chapter 3 (Table 4). These findings are consistent with suggestions made by Cheeke and Bronson (1979), Pond *et al.* (1991) and Bressani *et al.* (1992) that the

Fig.3. Effect of processed amaranth level and species

On chick body weight at 4 weeks of age



factors in amaranth were heat labile especially to moist heat. This is further supported by lack of differences ($P>0.05$) in chick body weight and pancreas weight for both *A. hypochondriacus* and *A. cruentus* species (Table 16).

Adverse effects of grain amaranth on chick performance appeared at the 60% level. The effects of remnants of antinutritional factors might have been substantial at this level especially with *A. cruentus* grain. As in Experiment 1, these factors might have interfered with the palatability, digestibility or utilisation of grain amaranth diets. In their study, Tillman and Waldroup (1988) reported that broilers could utilize up to 40% of heat treated *A. cruentus* without adverse effects on body weight and feed utilization. There was lower average mortality (5%) in this experiment compared to Experiment 1. The chicks mainly died in the first one week of age. Post mortem results showed the main cause of death was E. coli infection of the yolk sac.

5.4.4 Thigh meat composition

The mean moisture, protein and fat contents of the thigh meat are presented in Table 17. The moisture levels were lower while fat levels were higher than those reported in Experiment 1. This might have reflected the differences between normal and abnormal growth pertaining to chicks in Experiments 2 and 1 respectively. The fast growth rate of chicks fed on amaranth diets in Experiment 2 resulted in higher fat deposition. Diet 7 (60% *A. cruentus*) which had the

Table 17: Effect of heat processed amaranth on thigh meat composition at 4 weeks of age

Amaranth species	Control	A. hypochondriacus			A.cruentus		
Amaranth level, %	0	20	40	60	20	40	60
Diets	1	2	3	4	5	6	7
Moisture content % (NS)	71.56	71.93	72.27	72.29	72.04	71.94	72.98
Protein content % (NS)	18.73	18.95	18.49	18.82	18.80	18.56	18.64
Fat content % (NS)	8.49	7.81	8.17	8.04	8.11	8.33	7.40

NS = Means within a row are not different (P>0.05)

lowest ($P<0.05$) feed intake apparently gave lower fat and higher moisture content than the others. Restriction in feed intake is expected to reduce the growth rate and fat deposition in chicks as discussed in Experiment 1.

5.4.5 Histopathology

Results of microscopic evaluation of liver, pancreas and kidney tissues showed some degenerative changes similar to those described in Experiment 1. The lack of differences between treatments indicated that the observed changes were not caused by the different dietary treatments.

5.5 Conclusions

a) Heat treatment improved metabolisable energy of grain amaranth but lowered the crude protein content by about 1.40%.

b) Extruded grain amaranth can replace maize in broiler starter diets up to about 40%.

c) The two amaranth species had the same feeding value with respect to weight gain and feed efficiency.

d) Extruded grain amaranth had no effect on carcass composition and internal organs of broiler chicks.

CHAPTER 6

6.0 EXPERIMENT 3: SUPPLEMENTATION OF RAW AND THERMAL PROCESSED AMARANTH DIETS WITH CASEIN AND ESSENTIAL AMINO ACIDS

6.1 Introduction

The results of Experiment 1 showed that raw grain amaranth was poorly utilised by broiler chickens. Inclusion level beyond 40% was detrimental to the chicks performance, especially with *A. caudatus* and *A. cruentus*. However, results of Experiment 2 showed a marked improvement in broiler performance when the grain was heat processed. In both experiments, the diets were formulated to meet the energy, protein and essential amino acid requirements. It is evident that certain factors interfered with feed intake and utilisation of nutrients in the raw amaranth diets, and at the 60% level of the heat processed grain. A possible cause for the depressed broiler performance could be low availability of essential amino acids coupled with poor efficiency of utilisation.

In this experiment, amaranth (*A. hypochondriacus*) diets were fortified with synthetic lysine, methionine and purified casein. Broiler performance, nitrogen retention and amino acid availability were assessed. In literature, grain amaranth was reported to have a high level of phytic acid which interfered with availability of minerals in the gut.

Inorder to test this hypothesis, mineral retention was also measured.

6.2 Objectives

a) To determine the effect of supplementing raw and heat processed amaranth diets with synthetic essential amino acids and casein on broiler performance, amino acid availability and nitrogen retention.

b) To determine the effect of feeding raw and heat processed amaranth diets on mineral retention in broiler chickens.

6.3 Materials and methods

6.3.1 Experimental procedure

In the previous experiments raw and heat processed *A. hypochondriacus* species exhibited superiority with respect to low antinutritional factor content and high performance of broiler chicks. This grain species (variety 1024) was hence selected for this and subsequent experiments. Heat processing was carried out as described in Experiment 2.

A total of 288 day old 'Arbor acres' female chicks were obtained from a commercial hatchery. They were divided into groups of eight chicks each. Each group was weighed and put in one of 36 experimental pens. The size of the pen, type of litter used and general brooding management were as described in Experiment 1. On the 2nd day, every four pens were randomly assigned to one of nine experimental diets (Table 18). This gave nine dietary treatments with four replicates of eight

Table 18: Composition of diets used in experiment 4

Amaranth type	Control	Raw				Processed			
Amaranth level, %	0	40				60			
Amino acid/protein	None	None	Lysine	Lysine + Methionine	Casein	None	Lysine	Lysine + Methionine	Casein
Diet	1	2	3	4	5	6	7	8	9
Ingredients, %									
Maize	54.25	18.70	18.60	18.55	17.20	0.00	0.00	0.00	0.00
Amaranth	0.00	40.00	40.00	40.00	40.00	60.00	60.00	60.00	60.00
Corn oil	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Soya bean meal	30.00	30.00	30.00	30.00	30.00	30.00	30.00	30.00	30.00
Fish meal	8.00	3.50	3.50	3.50	3.50	2.13	2.20	2.15	0.75
Limestone	0.80	1.50	1.50	1.50	1.50	2.00	1.95	1.90	2.00
Dicalcium phosphate	1.20	0.55	0.55	0.55	0.55	0.12	0.00	0.00	0.00
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Vit./mineral premix+	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Lysine	-	-	0.10	0.10	-	-	0.10	0.10	-
Methionine	-	-	-	0.05	-	-	-	0.05	-
Casein	-	-	-	-	1.50	-	-	-	1.50
Determined analyses (air dry basis)									
TME, kcal /kg	3793.00	3701.00	3668.00	3685.00	3792.00	3850.00	3806.00	3826.00	3869.00
Dry matter, %	89.19	89.25	89.38	89.77	89.07	90.14	90.41	90.67	90.41
Crude protein, %	23.49	22.25	22.11	22.56	23.54	22.47	22.23	23.01	23.43
Ether extract, %	7.55	7.56	7.59	7.41	7.35	7.68	7.89	7.71	7.85
Crude fibre, %	5.48	6.21	6.07	6.79	6.77	6.54	6.67	7.06	6.69
Ash, %	6.07	6.14	6.28	6.10	6.44	5.94	5.70	5.93	5.57
Calcium, %	0.85	0.90	0.94	0.91	0.95	0.98	0.96	1.04	1.08
Phosphorus, %	0.72	0.78	0.81	0.79	0.75	0.81	0.78	0.80	0.86
Magnesium, %	0.25	0.30	0.31	0.29	0.32	0.33	0.35	0.34	0.36
Zinc, ppm	103.00	112.00	115.00	120.00	118.00	127.00	132.00	128.00	135.00

+Vitamin mineral premix provided per kg feed:- vitamin A, 1200 IU; vitamin D3, 300 IU; vitamin E, 10 IU;vitamin K, 4mg;vitamin B12,0.008mg; nicotinic acid, 20mg; pantothenic acid, 10mg; riboflavin, 6mg;folic acid, 2mg; biotin, 0.008mg; choline,0.30g; manganese, 62.5mg; zinc, 62.5mg; iodine, 1.5mg; copper, 6.25mg; selenium, 0.2mg; ethoxyquin, 9g

chicks each, in a completely randomised design. Diet 1 was a maize-soya bean meal control with no amaranth. Diets 2-5 had a range of 17.20-18.70% maize grain and 40% raw amaranth. Diets 6-9 contained no maize grain but had 60% heat processed amaranth.

Diet 1 contained the highest level of fish meal (8%) while diets 6-9 had the least (0.75-2.20%). The level of fish meal inclusion was consistent with the protein and amino acid content of maize and amaranth grains respectively. The increasing level of fish meal as the maize grain content increased was necessary to balance and meet the amino acid requirements. Diets 3 and 7 contained synthetic lysine included at 0.1% while each of diets 4 and 8 had lysine and methionine at 0.1 and 0.05% respectively. Diets 5 and 9 contained casein at a rate of 1.50%. This level of casein was aimed at providing the approximate amounts of lysine and methionine added to diets 3, 4, 7 and 8, in addition to other essential amino acids. Feed and water were provided *ad libitum*.

After an experimental period of 28 days, four chicks per pen were selected at random and placed in a metabolism cage designed for total faecal collection. The labelling of the cages corresponded to that of the pens to ensure continuation of the same dietary treatments and replicates. Measurements of body weight and feed per pen were taken. Clean, metal trays were placed under the cages to collect the excreta. After 72 hours, the trays were removed. Feathers and scales

were separated from the faecal droppings. The weights of birds and feed per cage were recorded. The faecal output was dried in an oven at 105°C and the dry weights recorded. The excreta was then finely ground in a Wiley mill with a 1mm sieve and analysed for crude protein, amino acids, calcium, phosphorus, magnesium and zinc, using methods described in Chapter 3.

Apparent nutrient retention or availability was calculated as follows:-

$$\text{Apparent nutrient retention (availability) \%} = \frac{\text{Wt. of nutrient ingested - wt. of nutrient in excreta}}{\text{Weight of nutrient ingested}} \times 100$$

For amino acids, apparent availability mainly reflected apparent digestibility and absorbability. This was because the amino acids absorbed and not utilised in the body were catabolised and excreted mainly as uric acid. Amino acids found in the excreta were thus an indicator of indigestibility of protein. Nitrogen retention indicated both undigested protein and unutilised amino acids (excreted as uric acid).

At the end of the experiment, one bird per replicate was sacrificed, its right thigh removed and the femur carefully obtained. The femur was boiled in water for a few minutes to remove any adhering soft tissue. It was then dried in the oven at 105°C for 24 hours, ground finely with a pestle and mortar and subjected to mineral analysis.

6.3.2 Data collection

Data on feed intake, weight gain and feed efficiency was recorded as described in Experiment 1. Chemical composition of diets and excreta, metabolisable energy of diets and mineral composition of the femur were determined as described in Chapter 3.

6.3.3 Statistical data analysis

This was carried out as described in Experiment 1 using a one way analysis of variance (SAS, 1985).

6.4 Results and discussion

6.4.1 Chemical composition of diets

The chemical analyses of diets used in Experiment 3 are presented in Table 18. The protein content of the diets ranged between 22.11 and 23.54% while the true metabolisable energy range was 3668-3869 kcal/kg of diet. The crude fibre levels were generally high at around 6%. This was caused by the use of a new batch of soya bean meal which contained more crude fibre than the one used in earlier experiments. The calcium and phosphorus contents of the diets were within the NRC (1984) specifications. Magnesium and zinc levels were in the range of 0.25-0.36% and 103-135 ppm, respectively. These levels were higher than the NRC (1984) requirements.

Amino acid content of the diets is presented in Table 19. Whereas the diets were formulated to contain adequate levels of essential amino acids, the determined composition showed

Table 19: Amino acid content of diets used in experiment 3

Amaranth type	Control	Raw				Processed			
Amaranth level, %	0	40				60			
Amino acid/Protein added	None	None	Lysine	Lysine + Methionine	Casein	None	Lysine	Lysine + Methionine	Casein
Diet	1	2	3	4	5	6	7	8	9
Amino acid, %									
Alanine	1.18	0.85	0.83	0.85	0.89	0.84	0.84	0.83	0.87
Arginine	1.30	1.25	1.24	1.26	1.29	1.28	1.30	1.29	1.37
Aspartic acid	2.18	1.95	1.89	1.96	2.02	1.86	1.84	1.82	1.88
Cystine	0.30	0.33	0.33	0.35	0.34	0.32	0.34	0.32	0.32
Glutamic acid	4.12	4.22	4.03	3.90	4.29	3.78	3.87	3.77	4.12
Glycine	0.98	0.95	0.93	0.94	0.95	1.04	1.06	1.03	1.05
Histidine	0.49	0.45	0.43	0.43	0.47	0.43	0.44	0.43	0.47
Isoleucine	0.60	0.61	0.54	0.50	0.65	0.54	0.60	0.58	0.71
Leucine	1.65	1.32	1.30	1.29	1.41	1.24	1.26	1.24	1.36
Lysine	1.17	1.04	1.08	1.08	1.10	1.05	1.18	1.13	1.15
Methionine	0.41	0.37	0.37	0.43	0.40	0.38	0.39	0.42	0.40
Phynylalanine	0.97	0.82	0.78	0.80	0.87	0.81	0.78	0.76	0.82
Proline	1.36	1.25	1.07	0.81	0.80	0.89	1.01	1.02	1.00
Serine	1.20	1.08	1.08	1.11	1.17	1.11	1.10	1.09	1.14
Threonine	0.83	0.73	0.70	0.70	0.77	0.72	0.70	0.71	0.76
Tyrosine	0.82	0.63	0.61	0.69	0.75	0.67	0.64	0.64	0.69
Valine	0.60	0.66	0.68	0.63	0.70	0.58	0.59	0.62	0.74

deficiencies especially for amaranth diets without casein. Valine levels in diets without casein ranged between 0.53-0.69% instead of the optimal 0.82%. Similarly, threonine in amaranth diets without casein was in the range of 0.70-0.73% instead of the recommended 0.80%. Isoleucine was slighly deficient in all the diets being in the range of 0.50-0.71% instead of the NRC (1984) level of 0.80%. Arginine levels in all the diets were between 1.20 and 1.37%, falling short of the NRC (1984) value of 1.44%. The range for methionine content was 0.37-0.43% while that of methionine plus cystine was 0.70-0.78%. Diets 4 and 8 with added methionine contained slightly higher levels of this amino acid than the others. The NRC (1984) requirement for methionine at 0-3 weeks of age is 0.50%, decreasing to 0.38% at 3-6 weeks of age. The requirement for methionine plus cystine is 0.93%. On the whole, the diets containing casein were superior to the other amaranth diets in amino acid content. However, the control diet had higher levels of threonine, leucine, phenylalanine, histidine and tyrosine compared with all the amaranth diets. The higher level of fishmeal in the control diet contributed to the adequate essential amino acid balance. According to Becker *et al* (1981) the first limiting amino acid in *A. cruentus* was leucine, followed by valine and threonine.

Some authors have reported optimum broiler performance using levels of essential amino acids below the requirement. Boorman (1992) found that weight gain in chicks was maximised at concentrations of 0.35% methionine or more. The same

author also gave recommendations of amino acid requirements from various sources which included lower values than those of NRC (1984). Koide *et al.* (1993) obtained maximum body weight gain and minimum feed conversion ratio with diets containing below NRC requirements for sulphur amino acids, lysine, threonine, arginine and tryptophan.

6.4.2 Broiler Performance

a) Body weight, feed intake and nitrogen retention

Results of chick body weight, feed intake, feed conversion efficiency and nitrogen retention are presented in Table 20. Chicks on the raw amaranth diets (2-5) had strikingly lower body weights than those on heat processed amaranth diets (6-9) and the maize soya bean meal control. Chicks on diets 1 (control) and 9 (60% processed amaranth with casein) had the highest ($P<0.05$) body weight. In the group containing raw amaranth, diet 5 with casein produced the highest body weight and nitrogen retention. Chicks on the processed amaranth diets gave similar ($P>0.05$) feed intake and nitrogen retention as the control diet. Although the difference was not significant ($P>0.05$), the trend indicated slightly lower values for the two parameters with processed amaranth diets. The feed intake for the raw amaranth diets (2-5) was lower ($P<0.05$) than that of the processed amaranth diets and that of the control.

Despite the variations in body weight and feed intake, all the nine diets gave similar feed efficiency. The results

Table 20: Effect of raw and processed amaranth diets on broiler performance at 31 days of age

Amaranth type	Control	Raw				Processed			
Amaranth level, %	0	40				60			
Amino acid/Protein added	None	None	Lysine	Lysine+ Methionine	Casein	None	Lysine	Lysine+ Methionine	Casein
Diet	1	2	3	4	5	6	7	8	9
Response									
Body weight g/bird	1090[a]	810[d]	778[d]	800[d]	832[cd]	955[bc]	942[bc]	957[bc]	1002[ab]
Feed intake, g/bird	1815[a]	1550[b]	1473[b]	1512[b]	1505[b]	1788[a]	1765[a]	1785[a]	1809[a]
Feed:gain	1.75[a]	2.04[a]	2.02[a]	2.02[a]	1.92[a]	1.98[a]	1.98[a]	1.97[a]	1.90[a]
Nitrogen retention (%)	70.71[a]	57.75[c]	57.25[c]	59.86[bc]	62.20[bc]	67.85[ab]	68.05[ab]	68.42[ab]	69.96[a]

[ab]Means with the same superscript within the row are not different (p>0.05).

of this study were in agreement with those of Waldroup *et al* (1985) who found depressed performance from broiler chicks on 40% raw amaranth diets. In addition, Tillman and Waldroup (1988c) found that 50% extruded amaranth diets gave poorer chick body weights than the maize control diet.

b) Amino acid availability (digestibility)

Results of apparent amino acid availability are presented in Table 21. The availability of essential amino acids in various diets was generally high, ranging between 73 and 95%. For the 11 amino acids recorded, the control diet gave the highest values. However, histidine was equally ($P>0.05$) available in all the diets. Arginine availability was also similar ($P>0.05$) in all the diets except diet 8. All the amaranth diets gave equal leucine availability which was lower than that of the control diet. Casein increased the availability of isoleucine and valine in the processed amaranth diet (9) making it similar ($P<0.05$) to that of the control diet. Processed amaranth diets gave better availability of isoleucine, valine and threonine than the raw grain diets.

The control diet was generally superior to the processed amaranth diets with respect to chick body weight (except for the casein diet) and amino acid availability. The high palatability and digestibility of maize grain and the high content (8%) and quality of fish meal in the control diet were the attributes that caused the superior chick performance.

Table 21: Apparent amino acid availability

Amaranth type	Control	Raw				Processed			
Amaranth level	0	40				60			
% Amino acid/ Protein added	None	None	Lysine	Lysine+ Methionine	Casein	None	Lysine	Lysine+ Methionine	Casein
Diet	1	2	3	4	5	6	7	8	9
Amino acid, %									
Arginine	95.19^{a}	93.35^{ab}	93.98^{ab}	93.58^{ab}	94.37^{ab}	93.89^{ab}	93.49^{ab}	92.24^{b}	94.35^{ab}
Cystine	89.59^{a}	86.74^{b}	85.78^{b}	86.23^{b}	87.75^{ab}	85.67^{b}	85.98^{b}	86.61^{b}	87.63^{ab}
Histidine	93.76^{a}	90.42^{a}	89.58^{a}	88.79^{a}	90.88^{a}	89.47^{a}	90.32^{a}	88.37^{a}	89.49^{a}
Isoleucine	88.34^{a}	83.26^{cd}	79.71^{de}	77.81^{e}	83.51^{cd}	83.65^{bcd}	85.10^{bc}	83.06^{cd}	88.43^{a}
Leucine	92.72^{a}	87.53^{b}	85.56^{b}	85.28^{b}	87.88^{b}	86.47^{b}	87.27^{b}	85.09^{b}	88.05^{b}
Lysine	92.17^{a}	86.67^{b}	86.50^{b}	86.03^{b}	86.87^{b}	86.14^{b}	88.21^{b}	85.46^{b}	87.57^{b}
Methionine	94.43^{a}	90.76^{b}	90.00^{b}	90.48^{b}	90.62^{b}	90.99^{b}	91.06^{b}	90.48^{b}	91.90^{b}
Phenylanine	89.56^{a}	83.45^{bc}	82.06^{bcd}	81.57^{cd}	84.19^{b}	83.75^{bc}	83.56^{bc}	80.89^{d}	84.20^{b}
Threonine	89.22^{a}	83.33^{bcd}	81.20^{cd}	80.62^{b}	83.92^{bc}	83.29^{bcd}	84.30^{bc}	81.84^{bcd}	84.96^{b}
Tyrosine	81.32^{a}	67.93^{b}	64.29^{b}	66.64^{b}	71.73^{b}	69.84^{b}	68.74^{b}	68.07^{b}	70.40^{b}
Valine	86.77^{a}	80.98^{bc}	77.60^{cd}	73.12^{cd}	81.98^{abc}	79.93^{bc}	82.91^{b}	79.41^{bc}	86.69^{a}

^{ab}Means with the same superscript within the row are not different (p>0.05).

Processed amaranth diets in turn exhibited a better chick performance than those containing raw amaranth. The performance differential between the control, the heat processed and the raw amaranth diets could be traced through the various stages of feed utilisation starting from ingestion.

The first difference observed was in feed consumption. Chicks on the control and processed amaranth diets consumed more than those on raw amaranth diets. The poor feed intake of the raw grain diets resulted in correspondingly low protein intake. This was then subjected to poor digestibility resulting in poor nitrogen retention. The result was a scarcity of amino acids that could be utilised for tissue synthesis and hence the low body weights observed. In addition to the dietary nitrogenous loss through poor digestibility, the antinutritional factors might have increased endogenous nitrogen output thus leading to the poor nitrogen retention. This would happen through the action of tannins and protease inhibitors complexing with digestive enzymes as discussed earlier in literature. The depression in feed intake with the raw amaranth diets appeared to have resulted from poor palatability or poor acceptability of the feed. This could be associated with the astringency caused by antinutritional factors such as tannins which bind with proteins on the tongue and in the oral cavity (Cheeke and Shull, 1985). Tannins also cause irritation and damage to the intestinal mucosa while trypsin inhibitor causes enlargement

of the pancreas (Huisman and Tolman, 1992). The total effect of these factors in raw amaranth was detrimental to the chick's gastro-intestinal tract and the digestive process. The possible presence of lectins and saponins in raw amaranth might have had a similar negative contribution on palatability and digestibility. The natural response from the chicks was rejection of such a feed as a regulatory mechanism to reduce the adverse effects. This observation was in agreement with that of Scott *et al.*, (1982) who found that chicks avoided certain feedstuffs probably on the basis of taste or adverse effects upon metabolism.

The processed amaranth diets were better than the raw grain diets with respect to feed intake, digestibility, nitrogen retention and weight gain. The improvement in performance was attributed to the destruction of antinutritional factors by the heat treatment. Although the processed diets were similar to the control diet in feed intake, they exhibited poorer amino acid availability and gave lower body weights compared to the control diet. The lower amino acid availability of the processed amaranth diets represented lower digestibility of dietary protein probably caused by heat processing and by any remaining antinutritional factors.

Fisher and Boorman (1986) noted that indigestibility accounted for most of the unavailability of amino acids. Heated protein was more resistant to digestive processes than the unheated one (Wallis and Balnave, 1984). Erbersdobler

(1976) explained that excessive heat treatment resulted in interactions between functional groups within the protein or with other food components like reducing sugars. Lysine, glutamic acid, aspartic acid and threonine were liable to this type of damage from heat treatment (Erbersdobler, 1976; Jondreville, 1994). An important interaction with other food components was the 'Maillard reaction' resulting in formation of a lysine-sugar complex which hindered the action of trypsin (Erbersdobler, 1976). Such interactions might have explained the significant differences in lysine and threonine availability between the control and the processed amaranth diets.

Addition of casein to the processed amaranth diet improved availability of some essential amino acids (Table 21). In addition, casein generally increased weight gain and nitrogen retention for the amaranth diets. The benefit was more apparent with the raw amaranth diet. This implied that casein protein was highly digestible and thus more available compared to the raw amaranth protein. Amaranth diets in this study, were generally deficient in methionine, threonine, isoleucine, valine, arginine and leucine to varying degrees (Table 19). Casein is rich in these amino acids at levels of about 2.8, 3.9, 5.4, 6.4, 3.3 and 8.7% respectively (Scott *et al*, 1982). Inclusion of casein corrected any dietary deficiencies of these essential amino acids, hence increasing their availability for utilisation. This resulted in higher chick body weights.

Addition of lysine plus methionine caused slight improvement in chick weight gain and nitrogen retention compared to addition of lysine alone. The improvement was probably related to the higher digestibility and absorption of the synthetic amino acids. The increase in dietary methionine (Table 19) was beneficial since the levels of this amino acid were generally lower than the NRC (1984) requirement in all the diets. Laovoravit (1986) found that chick growth increased proportionally with increasing dietary lysine content between 1.05 and 1.35% using autoclaved amaranth diets. Similarly, a significant reduction in growth occurred when the amaranth diets contained 0.78% methionine plus cystine in comparison to an NRC (1984) requirement of 0.93%.

c) Mineral Utilisation

Results of mineral retention and femur mineral content are presented in Table 22. The control and the processed amaranth diets gave the highest mineral retention. The mean retention values for calcium, phosphorus, magnesium and zinc were 35.28, 45.29, 32.77 and -0.86% for the raw amaranth diets and 49.61, 49.99, 31.01 and 6.14% for the processed amaranth diets. There were no differences ($P>0.05$) between dietary treatments in magnesium retention, femur magnesium, femur phosphorus and femur zinc.

The femur calcium and phosphorus levels were similar to those obtained by Pedersen *et al.*, (1987) after feeding amaranth diets to rats. Shafey *et al* (1990) obtained similar

Table 22: Effect of raw and processed amaranth diets on mineral utilisation at 4 weeks of age.

Amaranth type	Control	Raw				Processed			
Amaranth level, %	0	40				60			
Amino acid/Protein added	None	None	Lysine	Lysine + Methionine	Casein	None	Lysine	Lysine + Methionine	Casein
Calcium retention (%)	42.49^{cd}	36.49^{de}	34.64^{e}	35.62^{e}	34.36^{e}	45.91^{bc}	47.51^{bc}	51.20^{ab}	53.83^{a}
Phosphorus retention(%)	42.19^{b}	43.47^{b}	46.71^{ab}	45.09^{ab}	45.90^{ab}	50.82^{a}	50.21^{a}	48.96^{ab}	48.98^{ab}
Magnesium retention (%)	34.88^{a}	28.29^{a}	33.38^{a}	34.97^{a}	34.45^{a}	31.85^{a}	29.10^{a}	28.71^{a}	34.40^{a}
Zinc retention (%)	0.45^{bc}	-2.71^{c}	-1.92^{c}	0.25^{bc}	0.94^{bc}	3.46^{ab}	6.52^{ab}	6.33^{ab}	8.25^{a}
Femur calcium (%)	14.49^{a}	13.03^{b}	13.01^{b}	13.70^{b}	13.92^{b}	14.53^{a}	14.47^{a}	14.25^{a}	14.15^{a}
Femur Phosphorus (%)	7.40^{a}	7.49^{a}	7.43^{a}	7.45^{a}	7.39^{a}	7.25^{a}	7.32^{a}	7.28^{a}	7.71^{a}
Femur magnesium (%)	0.47^{a}	0.48^{a}	0.45^{a}	0.44^{a}	0.46^{a}	0.40^{a}	0.43^{a}	0.42^{a}	0.44^{a}
Femur zinc, ppm (%)	168^{a}	170^{a}	174^{a}	176^{a}	166^{a}	170^{a}	176^{a}	168^{a}	173^{a}

^{ab}Means with the same superscript within the row are not different ($p>0.05$).

tibia calcium (13.7-14.9%) and phosphorus (6.7-7.5%) for 17 days old broiler chicks. There was no evidence of deficiency in these minerals in chicks fed the amaranth diets. The higher calcium, phosphorus and zinc retention with the processed amaranth diets was probably partly influenced by a change in phytate solubility due to heat treatment. A more important factor that could have played a role in absorption was the higher digestibility and nitrogen retention for the processed diets. Amino acids are known to be effective organic chelates and could be of primary importance in the transport of mineral elements (Scott *et al*, 1982).

In the raw amaranth diets, higher endogenous losses of these minerals might have occurred through complex reactions of enzymes with antinutritional factors. The higher levels of femur calcium with the processed amaranth diets conformed with the higher calcium retention values for these diets.

Availability of phosphorus might have been influenced by the dietary source. Only a small (inorganic) part of the total plant phosphorus is fully available to birds. The majority which makes about 55-65% is in the phytin form (Simons, 1986). Birds are not able to utilize phytin phosphorus due to lack of phytase in the gastrointestinal tract (Scott *et al.*, 1982; Simons 1986). The femur magnesium levels were higher than those for bone magnesium reported by Scott *et al.* (1982). This was probably in response to the high dietary magnesium levels which exceeded the NRC (1984) specification of 600 ppm.

The low zinc retention was similar to that reported by Pedersen *et al.* (1987) who found low availability of zinc from amaranth products. In their study, the low intake groups of rats had a negative zinc balance. These authors found that processing of amaranth products had no effect on femur zinc although popping reduced phytate levels by 10-20%. The low zinc availability was attributed to the higher phytate: zinc ratios in amaranth (Pedersen *et al*, 1987).

The diets used in the present study had generally higher levels of zinc compared to the NRC (1984) specification of 40 ppm. Chicks exhibit a considerable tolerance to high intakes of zinc, up to 1400 ppm (Underwood, 1981). The low retention of zinc could partly be attributed to the high phytate levels of amaranth. Perdersen *et al.* (1987) reported that the quantity of phytate in amaranth seeds was 0.32-0.46% and that this content was about 1.5 times that of wheat. Nelson *et al.* (1968), Maga (1982) and Lorenz and Wright (1984) reported average phytate values in wheat of 0.20, 0.99 and 1.21% respectively. Similar values in maize were 0.17 and 0.90% respectively (Nelson, 1968; Lorenz and Wright, 1984). Pond *et al.* (1991) and Lorenz and Wright (1984) reported phytate levels in amaranth of 0.27-0.43% and 0.52-0.61% respectively. These conflicting findings show that the phytate level in amaranth grain is most likely similar to that of cereal grains. Phytate is known to form insoluble complexes with zinc thus interfering with availability of this mineral (Scott, *et al.*, 1982; Maga, 1982; Dewar, 1986).

The low zinc availability from the control diet in this study was probably associated with the high maize grain and soyabean meal contents of that diet. Savage *et al.* (1964) found impaired zinc absorption when soyabean protein was consumed by chicks. Maga (1982) reported higher phytate levels in soyabean meal (about 1.42%) than in cereals. In the current study, the femur zinc levels were relatively high (about 170 ppm) despite the low zinc retention.

The low bioavailability of zinc in high phytate diets has been investigated by various authors. Oberleas (1985) reported enhanced chemical complexation of zinc by phytate especially in the presence of excess calcium. The pancreas served an important function in the maintenance of zinc homeostasis since the major route for zinc excretion in the gut occurred through the pancreatic fluid (Oberleas, 1985). The raw amaranth diets used in the present study had normal levels of calcium. However, they might have provoked considerable losses of zinc through oversecretion of pancreatic enzymes.

6.5 Conclusions

a) Thermal treated grain amaranth at 60% inclusion level resulted in better chick performance than the 40% raw amaranth diets.

b) Chicks on the raw grain amaranth diets showed poor feed intake, poor amino acid digestibility and poor nitrogen retention.

c) Improvement in weight gain due to addition of casein, implied deficiency in some essential amino acids.

d) Lack of improvement from addition of lysine and methionine in amaranth diets implied the two amino acids were not limiting.

e) Although the zinc retention was poor for all the diets, the femur zinc content was adequate.

CHAPTER 7

7.0 EXPERIMENT 4: THE EFFECT OF ADDING CASEIN AND ETHYLENE DIAMINE TETRAACETATE (EDTA) TO RAW AND THERMAL PROCESSED AMARANTH DIETS.

7.1 Introduction

The results of Experiment 3 showed improved broiler performance when casein was added to raw and heat processed amaranth diets at a level of 1.50%. However, chicks on diets containing 60% heat processed amaranth did not perform as well as those on the maize control diet. It became necessary to test the effect of a higher casein level in a diet containing less processed amaranth. Results of Experiment 3 showed a low zinc retention especially with the raw amaranth diets. The phytate content of this grain might have interfered with zinc availability. Synthetic chelating agents such as EDTA can enhance availability of zinc and other mineral elements. In this experiment, EDTA and casein were separately added to the raw and heat processed amaranth diets.

7.2 Objectives

a) To study the effect of high levels of casein in amaranth diets on broiler performance, amino acid availability and plasma amino acids.

b) To determine the effect of EDTA on mineral utilisation in broilers fed amaranth diets.

7.3 Materials and methods

7.3.1 Experimental procedure

A total of one hundred and ninety two day old 'Arbor acres' broiler chicks were obtained from a commercial hatchery. On the second day, of age, they were randomly divided into 24 groups, each comprising eight chicks. Each group was assigned a pen and one of six experimental diets (Table 23). Each dietary treatment was replicated four times in a completely randomised design. The raw and heat processed grain amaranth used in this experiment was similar to that described in Experiment 3.

Diets 1-3 contained 40% raw amaranth grain while 4-6 had 40% thermal processed grain. Diets 2 and 5 contained disodium EDTA at a rate of 0.20%. Diets 3 and 6 had 3.0% casein each, aimed at providing about 0.2% lysine, 0.1% methionine and other essential amino acids. All the diets were generally similar with respect to other ingredients. The diets without casein contained high levels of fishmeal (6.00-6.50%) which were approximately double those found in Experiment 3 diets. This was done to ensure adequacy in essential amino acids, many of which were found wanting in diets of Experiment 3.

The brooding management was as described in Experiment 1. Feed and water were provided *ad libitum*.

At the end of four weeks of age, four chicks were selected at random from each replicate, weighed and put into the corresponding cage in the metabolism unit. The chicks were fed on the same diets as before for the next 72 hours.

Table 23: Composition of diets used in Experiment 4

Amaranth	40% Raw			40% Processed		
Supplement	None	EDTA	Casein	None	EDTA	Casein
Diet	1	2	3	4	5	6
Ingredients, %						
Maize	16.60	16.50	18.15	16.30	16.10	18.15
Amaranth	40.00	40.00	40.00	40.00	40.00	40.00
Corn oil	3.00	3.00	3.00	3.00	3.00	3.00
Soyabean meal	30.00	30.00	30.00	30.00	30.00	30.00
Fish meal	6.00	6.00	0.50	6.50	6.50	0.50
Limestone	1.50	1.40	1.80	1.40	1.40	1.80
Dicalcium Phosphate	0.15	0.15	0.80	0.05	0.05	0.80
Salt	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin/mineral premix[1]	2.50	2.50	2.50	2.50	2.50	2.50
Casein	-	-	3.0	-	-	3.0
EDTA	-	0.20	-	-	0.20	-
Determined Analyses[2]						
Dry matter, %	89.40	89.33	88.65	89.85	89.58	89.14
Crude protein, %	21.10	21.75	23.03	21.55	22.68	23.54
Ether extract, %	7.25	7.73	7.92	6.68	6.74	7.14
Crude fibre, %	5.33	5.42	5.08	5.86	5.39	5.17
Ash, %	6.08	6.53	6.54	6.50	6.61	6.72
TME, Kcal/kg	3621.00	3645.00	3684.00	3709.00	3753.00	3804.00
Calcium, %	1.24	1.18	1.31	1.29	1.22	1.44
Phosphorus, %	0.92	0.80	0.87	0.86	0.91	0.86
Magnesium, %	0.23	0.20	0.21	0.22	0.22	0.25
Zinc, ppm	103.00	89.00	86.30	79.30	107.00	90.00

1Vitamin mineral premix provided per kg feed:- vitamin A, 1200 IU; vitamin D_3, 300 IU; vitamin E, 10 IU; vitamin K, 4mg; vitamin B_{12}, 0.008mg; nicotinic acid, 20mg; pantothenic acid, 10mg; riboflavin, 6mg; folic acid, 2mg; biotin, 0.08mg; choline, 0.30g; manganese, 62.5mg; zinc, 62.5mg; iodine, 1.5mg; copper, 6.25mg; selenium, 0.2mg; ethoxyquin, 9g.
2Air dry basis

During this period, total faecal collection was carried out as described in Experiment 3. Feed intake, weight gain and dry faecal output were recorded.

At the end of the three days, blood samples were removed from the wing vein of the birds in each cage using heparinised syringes and gauge 19 needles. The blood was pooled together for the birds in a cage into a 10 ml heparinised test tube. It was then centrifuged at 3000g for 15 minutes and the plasma carefully separated. A few granules of sodium azide were added to the plasma to prevent microbial spoilage. Part of the plasma was stored at 4°C for immediate mineral analysis while the rest was frozen for subsequent amino acid analysis.

One bird per replicate was weighed and killed by cervical dislocation. Sections of the pancreas, and liver were obtained. The right thigh was removed after which the muscle tissue and femur were separately obtained. All the soft tissues collected were cut up separately and finely minced in a blender. The femur was boiled for 3 minutes and cleaned of adhering tissue, dried, weighed and then ashed in a furnace at 600°C. The ash and soft tissue samples were subjected to mineral analysis as described in Chapter 3. The blood plasma was directly analysed for minerals using atomic absorption spectroscopy and for amino acids using ionic exchange chromatography. In the latter, the plasma was first deproteinised using sulpho salicyclic acid. The pH was then adjusted to 2.2 with lithium hydroxide and thereafter analysed

(using lithium citrate buffers) according to the procedure described in Chapter 3.

7.3.2 Data Collection

This was as described in Experiment 1 for feed intake, weight gain and feed efficiency. Additional data was recorded on mineral content of diets, faecal output, pancreas, liver, thigh muscle, femur ash and plasma. Diet, faecal and plasma amino acids were determined. Apparent nitrogen, amino acid and mineral retention were calculated as described in Experiment 3.

7.3.3 Statistical data analysis

This was carried out as described in Experiment 1. The individual dietary treatments were compared and then the data was analysed as a 2 x 3 factorial (processing x supplementation).

7.4 Results and discussion

7.4.1 Chemical composition of the diets

The chemical composition of diets used in this experiment is presented in Table 23. The proximate and the mineral composition of the diets were generally within the NRC (1984) broiler chicken requirements. The protein content and the true metabolizable energy were in the range of 21.10-23.54% and 3621-3804 kcal/kg, respectively. There was little variation in the dietary crude fibre and ether extract

contents. The two ranged between 5.08-5.86% and 6.68-7.92%, respectively. This was expected due to the similarity in ingredient composition. However, an increasing trend was observed in metabolizable energy of the heat processed amaranth diets compared with those of the raw grain. This was attributed to higher digestibility of these diets compared to the raw amaranth diets.

The calcium and phosphorus levels were 1.18-1.44% and 0.80-0.92%, respectively. The magnesium and zinc contents were 0.20-0.25% and 79.30 - 107 ppm, respectively, thus exceeding the NRC (1984) requirements.

The determined amino acid composition of the diets is presented in Table 24. All the diets generally met the NRC (1984) specifications for amino acids. However, the methionine content which should be 0.50% (NRC, 1984) ranged between 0.34-0.43% in all the diets. The level of isoleucine in diets without casein was in the range of 0.65-0.76 instead of the required 0.80%. On the whole, diets with casein contained higher amino acid levels than the others.

7.4.2 Broiler performance

Results of mean body weight, feed intake, feed efficiency and nitrogen retention of the chicks at 31 days of age are presented in Table 25. The mean body weight was 954 grams, a value greater than that of NRC (1984) for 4 week old broiler chicks. Correspondingly, the cumulative feed intakes were also higher. Chicks on processed amaranth diets were heavier

Table 24: Amino acid composition of the diets used in experiment 4

Amaranth	40% Raw			40% Processed		
Supplement	None	EDTA	Casein	None	EDTA	Casein
Diet	1	2	3	4	5	6
Amino acid, %						
Alanine	0.89	0.89	0.97	0.94	0.94	0.97
Arginine	1.32	1.44	1.45	1.39	1.36	1.41
Aspartic acid	2.06	1.99	2.18	2.10	2.08	2.24
Cystine	0.35	0.35	0.40	0.36	0.36	0.38
Glutamic acid	4.35	4.27	4.81	4.46	4.39	4.71
Glycine	1.01	1.02	1.05	1.02	1.00	1.06
Histidine	0.48	0.49	0.53	0.50	0.48	0.51
Isoleucine	0.65	0.76	0.84	0.74	0.65	0.79
Leucine	1.42	1.40	1.56	1.46	1.41	1.52
Lysine	1.12	1.14	1.28	1.20	1.19	1.29
Methionine	0.37	0.38	0.40	0.35	0.34	0.43
Phenylalanine	0.85	0.91	0.97	0.96	0.93	1.02
Proline	1.48	1.20	1.52	1.25	1.35	1.25
Serine	1.13	1.08	1.21	1.17	1.18	1.22
Threonine	0.78	0.79	0.88	0.82	0.78	0.86
Tyrosine	0.62	0.70	0.78	0.74	0.73	0.80
Valine	0.83	0.82	0.92	0.80	0.76	0.82

Table 25: Broiler performance at 31 days of age

Diet	Body weight g/bird	Feed intake g/bird	Feed efficiency Feed:gain	Nitrogen retention, %
40% raw amaranth	863[c]	1528[b]	1.88[a]	64.01[b]
40% raw amaranth + EDTA	880[c]	1566[b]	1.89[a]	66.19[b]
40% raw amaranth + casein[1]	903[c]	1576[b]	1.85[a]	66.98[b]
40% Processed amaranth	983[b]	1689[ab]	1.81[a]	72.51[a]
40% Processed amaranth + EDTA	993[b]	1736[a]	1.84[a]	70.15[ab]
40% Processed amaranth + casein[1]	1070[a]	1783[a]	1.75[a]	72.68[a]

[1]Inclusion of casein increased ($P<0.05$) body weight compared to either EDTA inclusion or no inclusion.

($P<0.05$) than those on raw amaranth diets (Table 25; Figure 4). They also had higher ($P<0.05$) feed intake and nitrogen retention than those on raw amaranth diets. This indicated higher palatability and digestibility for the processed amaranth diets. The difference in feed efficiency between the two types of diets was not significant ($P>0.05$).

Inclusion of casein in diets 3 and 6 increased ($P<0.05$) body weight in comparison with diets containing EDTA or without any supplementation (Table 25; Figure 4). Similarly, the casein containing diets had generally higher feed intake and better feed efficiency than the others.

Casein increased the methionine content of diets 3 and 6 such that they contained higher levels of this amino acid than the rest of the diets (Table 24). In addition, the isoleucine content of these diets met the NRC (1984) requirement while that of the other diets did not. The adequate levels of these two essential amino acids might have boosted the utilisation of the casein containing diets, resulting in the observed higher chick body weights.

Availability of amino acids in chicks at 29-31 days of age is shown in Table 26. The availabilities were generally high and there were no differences ($P>0.05$) between diets. Thermal processing of the grain or casein inclusion did not ($P>0.05$) affect availability. In studies like these, it has been argued that lack of difference between dietary treatments could be due to factors affecting accuracy of faecal analysis (McNab, 1980). Ebersdobler (1976) and McNab (1980) reported

Fig.4. Effect of supplementation and processing on chick performance at 31 days of age

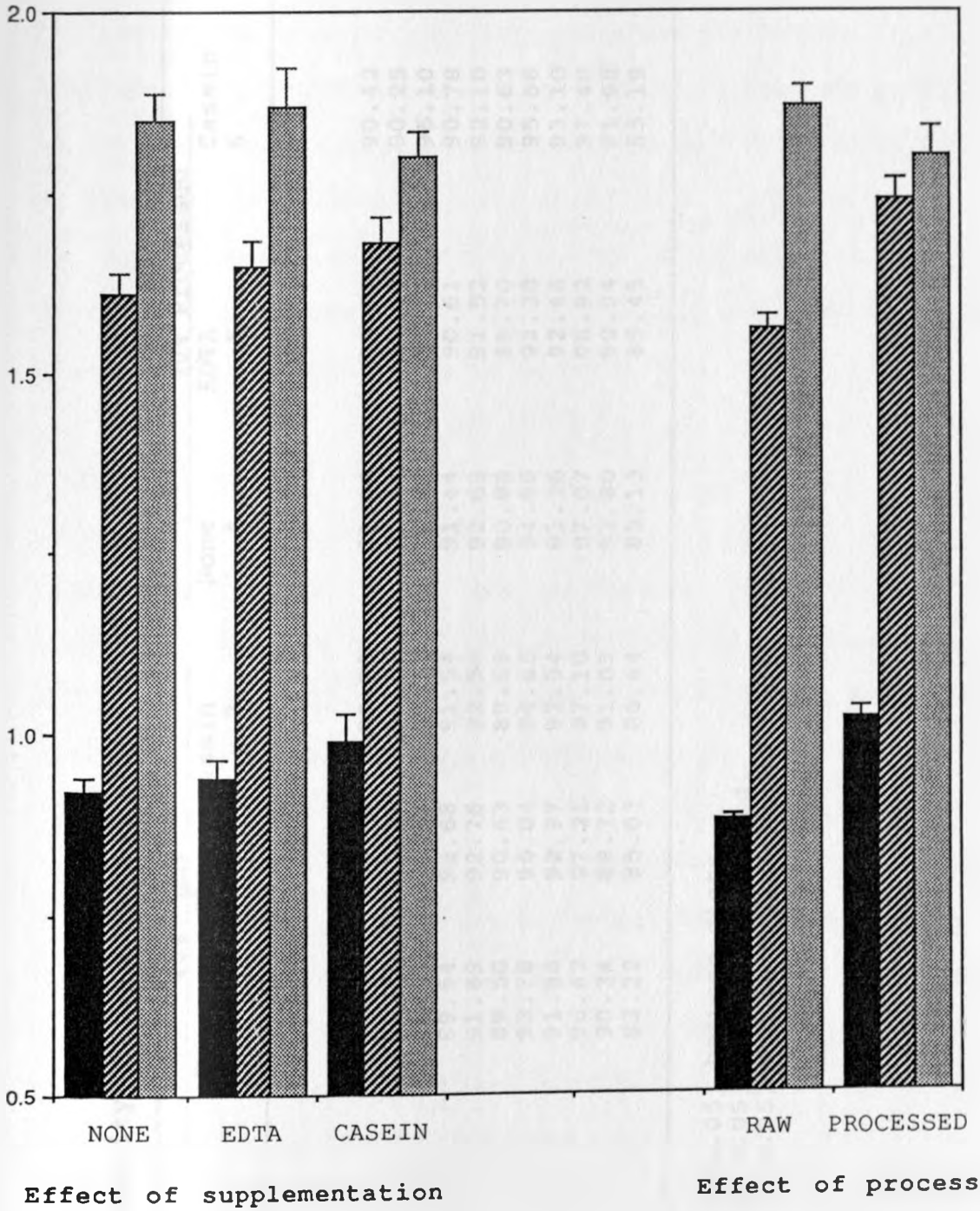


Table 26: Availability[1,2,3] of essential amino acids

Amaranth	40% Raw			40% Processed		
Supplement	None	EDTA	Casein	None	EDTA	Casein
Diet	1	2	3	4	5	6
Amino acid, %						
Threonine	89.66	90.81	90.90	91.36	89.94	90.42
Valine	88.58	91.27	90.37	90.54	88.68	90.25
Methionine	93.03	93.87	93.51	94.45	94.14	95.10
Isoleucine	89.54	92.68	91.54	91.44	90.61	90.78
Leucine	91.89	92.76	92.56	92.63	91.52	92.10
Phenylalanine	89.50	90.43	89.59	90.96	89.70	90.63
Histidine	93.78	95.04	94.65	94.86	93.38	95.56
Lysine	91.88	92.97	92.94	93.36	92.48	93.10
Arginine	96.62	97.21	97.10	97.07	96.92	97.40
Cystine	90.34	89.72	91.03	92.80	90.94	91.95
Tyrosine	83.32	85.03	86.64	85.13	85.45	85.19

[1]No differences (P>0.05) between diets
[2]No differences (P>0.05) due to processing.
[3]No differences (P>0.05) due to addition of EDTA or casein

that microflora in the lower gut might deaminate the undigested amino acid residues resulting in higher apparent availability. Nonetheless, in this study, amino acid profiles of the diets were similar, intuitively expecting similar level of availability. Despite the lack of significant differences in amino acid availability, it is likely that poor digestibility of raw amaranth diets mainly contributed to the reduced nitrogen retention of these diets.

Plasma amino acids of chicks at 31 days of age are presented in Table 27. There were no differences ($P<0.05$) between the various dietary treatments. The uniformity in plasma amino acid levels was in keeping with that of the dietary amino acid composition. The levels of plasma amino acids were similar to those obtained by Hewitt and Lewis (1972a and 1972b) in their determination of amino acid requirements of the growing chick. These authors (1972a) found elevated total plasma amino acid levels when the dietary lysine concentration was either inadequate or excessive. In their study, plasma levels were least with diets containing 0.9-1.15% in a 21% protein diet. The authors indicated that efficient utilization of dietary protein was closely related to relatively low levels of plasma amino acids.

The plasma amino acid levels in the current study generally fell within those obtained by the above mentioned authors for a dietary lysine range of 1.1-1.2%. A few exceptions were aspartic acid, serine and glycine levels which were slightly higher and tyrosine content which was lower than

Table 27: Plasma amino acids[1,2] at 31 days of age

Amaranth	40% Raw			40% Processed		
Supplement	None	EDTA	Casein	None	EDTA	Casein
Diet	1	2	3	4	5	6
Amino acid, (μmoles/ml)						
Alanine	0.78	0.83	0.75	0.76	0.70	0.67
Arginine	0.47	0.50	0.45	0.48	0.43	0.43
Aspartic acid	0.19	0.16	0.19	0.23	0.20	0.10
Cystine	0.07	0.05	0.05	0.05	0.05	0.04
Glycine	0.76	0.77	0.64	0.77	0.67	0.61
Histidine	0.15	0.17	0.19	0.14	0.12	0.16
Isoleucine	0.17	0.15	0.16	0.17	0.17	0.18
Leucine	0.25	0.23	0.25	0.22	0.25	0.25
Lysine	0.64	0.54	0.70	0.66	0.61	0.76
Methionine	0.07	0.06	0.06	0.07	0.07	0.08
Phenylalanine	0.15	0.14	0.15	0.14	0.11	0.11
Serine	0.77	0.76	0.86	0.80	0.93	0.83
Threonine	0.71	0.73	0.63	0.80	0.78	0.61
Tyrosine	0.16	0.15	0.16	0.15	0.14	0.16
Valine	0.31	0.28	0.30	0.30	0.30	0.26

[1]There were no differences (P>0.05) between the six dietary treatments.
[2]There were no differences (P>0.05) between the treatments of raw vs processed amaranth; or casein vs EDTA vs no supplement.

the values given by the same authors. The dietary lysine in this experiment ranged between 1.12 and 1.31%.

Plasma amino acid levels are a sensitive index of changes in the amino acid balance of the dietary supply (Hewitt and Lewis, 1972a). The diets used in the present study provided an adequate and well balanced supply of amino acids as reflected in the plasma amino acids. In conformity with the high availability values discussed earlier, these amino acids were optimally utilized to produce the high body weights observed. According to McNab (1980), plasma amino acid levels were a reflection of those in the protein undergoing digestion and being made available to the animal

7.4.3 Mineral utilization

Table 28 shows the mineral utilization with respect to retention and tissue composition. The retention rates for calcium, phosphorus, magnesium and zinc were higher ($P<0.05$) for the heat processed amaranth diets compared with the unprocessed ones. Zinc availability was actually negative for all the diets. The mineral content of various body tissues did not show striking differences between the processed amaranth diets and the unprocessed ones. The femur calcium, phosphorus, magnesium and zinc contents were similar ($P>0.05$). The same case applied to the magnesium content of the liver, pancreas, thigh muscle and plasma. However, the zinc contents of the liver, pancreas and plasma were lower ($P<0.05$) with the raw amaranth diets. This was in conformity with the lower

Table 28: Mineral utilisation at 31 days of age

Main effects		Processing		Supplementation		
		Raw	Processed	None	EDTA	Casein
Mineral Retention, (%)						
	Calcium	48.56[b]	54.25[a]	51.80	51.68	50.74
	Phosphorus	47.86[b]	53.58[a]	47.18	51.29	50.30
	Magnesium	43.15[b]	48.31[a]	45.25	46.48	45.47
	Zinc	-0.038[b]	-0.027[a]	-0.030	-0.033	-0.035
Tissue Mineral Content						
Femur(ash):	Calcium, %	36.80	37.50	36.28	37.37	37.81
	Phosphorus,%	18.88	19.42	18.54	19.32	19.60
	Magnesium,%	1.88	1.42	1.38	1.39	1.43
	Zinc,ppm	460.89	466.00	467.00	462.50	460.83
Thigh muscle:	Magnesium ,%	191.36	200.27	192.27	201.14	194.04
	Zinc,ppm	22.19	20.50	20.32	22.20	21.51
Liver:	Magnesium, %	379.40	374.27	372.30	380.53	377.67
	Zinc,ppm	34.55[b]	42.39[a]	37.90	37.72	26.53
Pancreas:	Magnesium,%	452.46	450.02	442.15	459.93	451.65
	Zinc,ppm	39.47	49.52	41.80	45.60	46.09
Plasma:	Calcium, mg%	10.21	9.77	9.64	10.03	10.30
	Phosphorus, mg%	5.98[b]	6.64[a]	5.98	6.51	6.45
	Magnesium, mg%	3.15	3.40	3.32	3.29	3.23
	Zinc, ppm	2.69[b]	3.00[a]	2.83	3.03	2.68

[ab]Means with different superscripts for each main effect within a row are different ($p<0.05$)

($P<0.05$) zinc retention for these diets.

The trend of higher mineral retention for the heat processed amaranth diets corresponded with that of nitrogen retention implying a positive relationship. Amino acids serve as chelating agents for minerals. The low zinc retention signified the presence of a factor(s) interfering with absorption of this mineral which was adequate in the diet. In Experiment 3, the phytate content was identified as the possible factor. Despite the negative zinc balance, the plasma and tissue zinc contents were normal and similar to those obtained by Watkins and Southern (1993) using maize-soyabean meal diets containing 85ppm zinc. This demonstrated a similarity between amaranth and maize grain with respect to zinc availability.

Underwood (1981) noted that diets containing plant protein sources such as soyabean meal could not be relied on to provide sufficient zinc for chicks because the chelating effects of these feeds on zinc induced higher requirements. The same authors reported that diets containing animal protein sources were unlikely to present zinc problems in poultry. The zinc levels of the soft tissues in the present study were similar to or higher than those reported by Scott *et al.* (1982) on fresh tissue basis. The chick tries to maintain the normal zinc concentration in its tissues even when deprived of this mineral in the diet (Savage *et al.* 1964; Scott *et al.* 1982). This is achieved through depletion of femur zinc since bone serves to store excess zinc which can be released for use

by other critical tissues. Pedersen (1987) reported that rats that had negative zinc balance when fed amaranth diets were unable to maintain their femur zinc concentration during the study period. In the present experiment, the femur zinc level was determined only once at the end of the study period. It was therefore not possible to ascertain whether there was depletion of the femur zinc concentration within a given time period. All the diets contained both plant and animal protein sources, and had above normal zinc requirements. This could explain the normal zinc levels in all the tissues despite the low retention rate.

The dietary phytate might also have played a homeostatic role in regulation of zinc turnover. Hallmans *et al* (1985) reported that dietary phytate and fibre act as a zinc reserve when the need increases but offer resistance to free absorbtion of the mineral when its concentration exceeds the body's need. If excess zinc is absorbed from low phytate diets, it causes increased excretion of endogenous zinc and increased accretion of the mineral in bones (Hallmans *et al*, 1985).

The magnesium levels of the femur, soft tissues and plasma for all the diets were within the normal range (Scott *et al.*, 1982). The plasma calcium and phosphorus levels were however slightly higher than those given by Scott and associates. These results indicate that the levels and availability of all the four minerals in the amaranth diets were adequate for normal mineral and body metabolism. Any

differences between the heat processed and raw amaranth diets were not large enough to cause adverse effects in mineral utilization for the latter diets.

Inclusion of EDTA in diets 2 and 5 did not enhance mineral retention. There were no differences ($P>0.05$) in mineral utilization between the diets with and without EDTA. This did not agree with work reported by Odell *et al* (1964) and Scott *et al*. (1982) whereby EDTA improved absorption and utilization of zinc in chicks. The phytate content of the amaranth diets was probably not high enough to cause a marked effect when EDTA was used. Availability of zinc in amaranth diets was not a limiting factor as observed from the adequate tissue content of this mineral.

7.5 Conclusions

a) Addition of casein in grain amaranth diets improved weight gain ($P<0.05$) due to improvement in dietary essential amino acid levels.

b) On the whole, chicks on heat processed amaranth diets gave better performance than those on raw amaranth diets. Amino acid availability was similar for both types of diets.

c) Plasma amino acid levels did not reflect excess or deficiency of essential amino acids in amaranth diets.

d) Inclusion of EDTA did not enhance mineral retention implying that availability of inorganic elements is not a limitation in using amaranth in poultry diets.

CHAPTER 8

8.0 EXPERIMENT 5: EFFECT OF PELLETING AMARANTH DIETS WITH OR WITHOUT MOLASSES ON BROILER PERFORMANCE AND CARCASS COMPOSITION

8.1 Introduction

In the preceding experiments, poor chick performance from raw amaranth diets was mainly caused by low feed intake resulting from poor acceptability of raw grain amaranth. It would be worthwhile to evaluate the inclusion of a flavouring agent in amaranth diets. Thermal processing of feed by extrusion has considerable cost implications and is not commonly done in Kenya. There is need to investigate the efficacy of a cheaper and more widely used feed manufacturing process such as steam pelleting. In Experiment 1, the chicks on raw amaranth diets underwent a faster growth rate after four weeks of age. This apparent adaptation needs to be investigated further. All these factors were considered in designing the nature of treatments in this experiment.

8.2 Objectives

a) To determine the effect of molasses as a flavouring agent in amaranth diets fed to broiler chickens.

b) To determine the effect of pelleting amaranth diets on broiler performance and carcass composition.

c) To investigate the effect of age of chicks on utilisation of amaranth diets.

d) To assess the effect of mash and pelleted amaranth diets on body tissues of broiler chicks at 4 and 8 weeks of age.

8.3 Materials and methods

8.3.1 Experimental procedure

A. hypochondriacus (1024) grain was obtained from the same source described in Experiment 1. Cane molasses was obtained from the local suppliers. Steam pelleting of diets was carried out at Unga Feeds Ltd, Nakuru. A California Press[1] type pelleting machine was used. The pelleting die had round holes, 5mm in diameter. The pelleting temperature was about 70°C and steam was applied at a pressure of 5.1 kg/cm². The pellets were then crumbled to a smaller size suitable for chick feeding.

Day old 'Arbor acres' female chicks were obtained from a commercial hatchery. They were fed on a commercial broiler starter for six days. On the seventh day, two hundred and eighty eight chicks were selected and divided into thirty six groups of eight chicks each. Each group was weighed, put in a pen and randomly assigned to one of nine dietary treatments (Table 29). The chicks were fed on the experimental diets up to eight weeks of age. There were four replicates (pens) per treatment in a completely randomised design. Diets 1-4 had

[1]Made by Simon Barron Ltd, England.

Table 29: Composition of diets used in experiment 5

Amaranth, %	20				40				0
Molasses	-M[1]	-M	+M	+M	-M	-M	+M	+M	-M
Pelleting	+P[2]	-P	+P	-P	+P	-P	+P	-P	-P
Diet	1	2	3	4	5	6	7	8	9 (Control)
Ingredients, %									
Maize	35.15	35.15	32.10	32.10	16.70	16.70	13.50	13.50	54.25
Amaranth	20.00	20.00	20.00	20.00	40.00	40.00	40.00	40.00	00.00
Corn oil	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Soyabean meal	30.00	30.00	30.00	30.00	30.00	30.00	30.00	30.00	30.00
Fish meal	7.50	7.50	7.50	7.50	6.00	6.00	6.20	6.20	8.00
Limestone	1.00	1.00	1.00	1.00	1.40	1.40	1.40	1.40	0.80
Dicalcium phosphate	0.60	0.60	0.65	0.65	0.15	0.15	0.15	0.15	1.20
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Vit./mineral premix[3]	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Molasses	-	-	3.00	3.00	-	-	3.00	3.00	-
Determined Analyses (air dry basis)									
Dry matter, %	89.53	90.05	89.12	89.19	89.36	90.78	89.41	89.65	90.95
TME, kcal/kg	3750.00	3714.00	3667.00	3629.00	3804.00	3762.00	3710.00	3685.00	3789.00
Crude protein, %	22.65	23.26	23.05	22.87	24.17	23.96	22.93	23.10	23.65
Ether extract, %	6.49	7.06	7.16	6.95	7.20	6.92	6.88	7.13	7.35
Crude fibre, %	5.26	5.21	5.48	5.46	5.74	5.80	5.66	5.83	4.17
Ash, %	5.98	5.90	5.89	6.12	6.55	6.69	6.93	6.35	5.20

[1]-M=without molasses; +M=with molasses
[2]+P=Pelleted diet; -P=not pelleted (mash diet)
[3]Vitamin mineral premix provided per kg feed:- vitamin A, 1200 IU; vitamin D_3, 300 IU; vitamin E, 10 IU; vitamin K, 4mg; vitamin B_{12}, 0.008mg; nicotinic acid, 20mg; pantothenic acid, 10mg; riboflavin, 6mg; folic acid, 2mg; biotin, 0.08mg; choline, 0.30g; manganese, 62.5mg; zinc, 62.5mg; iodine, 1.5mg; copper, 6.25mg; selenium, 0.2mg; ethoxyquin, 9g.

20% amaranth while 5-8 had 40%. Diets 3, 4, 7 and 8 contained molasses. Diets 1, 3, 5 and 7 were pelleted while 2, 4, 6 and 8 were in mash form containing raw grain amaranth. Diet 9 was a mash maize-soyabean meal control diet with no amaranth and no molasses. Feed and water were provided *ad libitum*. Diets used in this experiment were screened for mycotoxin contamination. Brooding management of the chicks was as described in Experiment 1.

At the end of the 4th and 8th weeks of age, 1 bird per replicate was sacrificed, weighed and its pancreas removed and weighed. Sections of the pancreas and liver were put in 10% formalin to be processed for histopathological examination. At the end of eight weeks of age, the birds left were weighed and then starved for 24 hours, to be used for carcass composition. Two birds in each replicate were sacrified, weighed, defeathered and weighed again. They were then cut into small sections and minced thoroughly using a large hand mincer. The minced meat was uniformly mixed and small random samples taken for protein, ether extract, ash and moisture analysis. The samples were minced fine in a blender.

8.3.2 Data collection

Data on weekly body weight, feed intake and feed conversion efficiency was recorded as described in Experiment 1.

The percentage of nutrient retained in the carcass was calculated as:-

$$\frac{\text{Amount of nutrient in carcass}}{\text{Amount of nutrient consumed}} \times 100$$

8.3.3 Statistical data analysis

This was carried out as described in Experiment 1. After comparing data for the 9 dietary treatments, the control was omitted and the remaining 8 treatments analysed as a 2 x 2 x 2 factorial.

8.4 Results and discussion

8.4.1 Chemical composition of the diets

The chemical composition of diets used in this experiment is presented in Table 29. The crude protein content ranged between 22.5 to 24.17% while the true metabolizable energy of the diets was 3629-3804 kcal/kg respectively.

The ether extract and ash contents of the diets ranged between 6.49-7.35% and 5.20-6.93% respectively. The crude fibre content of the amaranth diets ranged between 5.21-5.83% while the maize control diet had 4.17%. This is consistent with the higher crude fibre levels of amaranth compared to maize grain.

While pelleting appeared to increase the metabolizable energy content of the diets, inclusion of molasses caused a slight decrease. Cane molasses has a metabolizable energy of about 1960 kcal/kg compared to maize with about 3370 kcal/kg (Scott *et al*, 1982).

8.4.2 Broiler performance

Results of broiler performance weights at 4 and 8 weeks of age are presented in Tables 30, 31 and 32. The body weights at 4 weeks of age were in the range of 633-808 grams per chick (Table 30). The mean body weight of chicks on amaranth based diets was 720 grams. This was below the mean NRC (1984) weight (790g) for a four week old broiler. The chicks on pelleted diets grew faster ($P<0.05$) than those on the mash diets (Table 32). Similarly, chicks on diets containing amaranth at 20% level had a higher ($P<0.05$) body weight than those on 40% amaranth. The mean feed intake per chick (1-4 weeks of age) on amaranth diets was 1242 grams. There were no differences ($P>0.05$) in feed intake between diets.

Chicks on the 20% amaranth level diets had a better ($P<0.05$) feed efficiency than the 40% level.

The body weights at 8 weeks of age were in the range of 2148-2439 grams per chick (Table 31). The mean body weight for broilers on amaranth diets was 2295 grams which exceeded the mean NRC (1984) weight (2060g) for broilers of similar age. There were no differences ($P>0.05$) between the nine diets. When the pelleted diets were however compared with the mash diets, they gave higher ($P<0.05$) body weight (Table 32). The level of amaranth had no effect ($P>0.05$) on body weight at this age (Table 32).

No differences ($P>0.05$) occurred in feed intake which ranged between 4870-5042 grams per broiler. The general

Table 30: Broiler performance at 4 weeks of age

	20% raw amaranth in diet				40% raw amaranth in diet				Control
	without molasses		with molasses		without molasses		with molasses		Without molasses
Diet	Pelleted 1	Mash 2	Pelleted 3	mash 4	Pelleted 5	Mash 6	Pelleted 7	Mash 8	Mash 9
Body weight (g/bird)	755^{ab}	720^{abc}	798^{a}	735^{abc}	736^{abc}	633^{c}	745^{ab}	648^{bc}	808^{a}
Feed intake (g/bird)	1280^{a}	1221^{a}	1245^{a}	1270^{a}	1228^{a}	1201^{a}	1237^{a}	1230^{a}	1291^{a}
Feed efficiency (feed:gain)	1.88^{ab}	1.89^{ab}	1.72^{b}	1.92^{ab}	1.86^{ab}	2.15^{a}	1.85^{ab}	2.15^{a}	1.76^{b}
Pancreas weight (% body weight)	0.33^{b}	0.39^{ab}	0.34^{b}	0.36^{ab}	0.36^{ab}	0.42^{a}	0.32^{b}	0.38^{ab}	0.32^{b}

^{ab}Means with the same superscript within the row are not different (P>0.05).

Table 31: Broiler performance at 8 weeks of age

	20% raw amaranth in diet				40% raw amaranth in diet				Control
	without molasses		with molasses		without molasses		with molasses		Without Molasses
Diet	Pelleted 1	Mash 2	Pelleted 3	Mash 4	Pelleted 5	Mash 6	Pelleted 7	Mash 8	Mash 9
Body weight (g/bird)	2365[a]	2228[a]	2435[a]	2305[a]	2353[a]	2148[a]	2362[a]	2162[a]	2439[a]
Feed intake (g/bird)	5022[a]	4872[a]	5017[a]	4885[a]	4880[a]	4877[a]	4870[a]	4867[a]	5042[a]
Feed efficiency (Feed:gain)	2.15[b]	2.22[ab]	2.08[b]	2.15[b]	2.10[b]	2.31[a]	2.09[b]	2.29[a]	2.13[b]
Pancreas weight, 4 wks of age (% body weight)	0.33[b]	0.39[ab]	0.34[b]	0.36[ab]	0.36[ab]	0.42[a]	0.32[b]	0.38[ab]	0.32[b]
Pancreas weight, 8 wks of age, (% body weight)	0.18[a]	0.22[a]	0.20[a]	0.21[a]	0.20[a]	0.21[a]	0.19[a]	0.20[a]	0.19[a]

[ab]Means with the same superscript within a row are not ($p>0.05$)different.

Table 32: Effect of amaranth level, inclusion of molasses and pelleting of diets on broiler performance at 4 and 8 weeks of age

Performance	Body weight		Feed intake		Feed Efficiency		Pancreas wt.	Pancreas wt.
Age (weeks)	4	8	4	8	4	8	4	8
Mash vs pelleted	*	*	NS	NS	*	*	*	NS
Molasses vs No molasses	NS	NS	NS	NS	NS	NS	NS	NS
Pelleting X Molasses	NS	NS	NS	NS	NS	NS	NS	NS
Level (20 vs 40%)	*	NS	NS	NS	*	*	NS	NS
Pelleting x Level	NS	NS	NS	NS	NS	*	NS	NS
Molasses x Level	NS	NS	NS	NS	NS	NS	NS	NS
Pelleting x Molasses x Level	NS	NS	NS	NS	NS	NS	NS	NS

*Significant ($P<0.05$).
NS - Not significant ($P>0.05$).

pattern however indicated a higher intake with the pelleted diets compared to those in mash form and a higher intake at 20% compared with 40% amaranth inclusion. The feed efficiency followed a similar trend but being better ($P<0.05$) with the pelleted diets and those containing 20% amaranth (Table 32). The significant interaction between pelleting and amaranth level indicated that feed efficiency was enhanced by pelleting the diets containing 20% amaranth level compared to those with 40%.

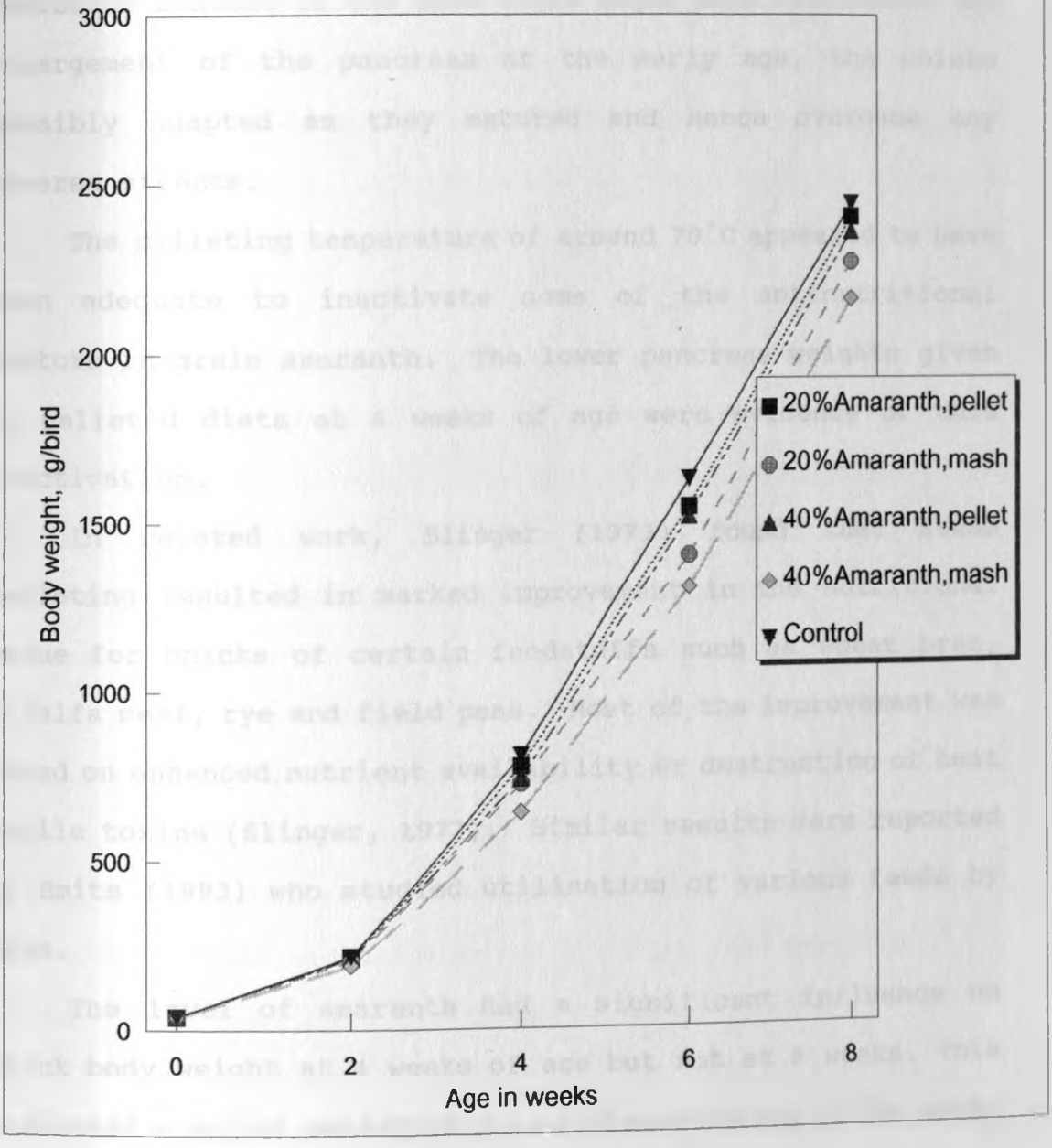
Figure 5 shows the growth curves of chicks on the various dietary treatments. The trend indicated faster growth for chicks on pelleted diets compared to those on mash diets. Chicks on 20% amaranth level diets also grew faster than those on diets containing 40% level (Figure 5).

The above results indicated that pelleting the diets improved their utilization. Similar results have been reported by Leclerq (1986) and Sengor and Bayne (1991) who found that pelleting increased energy intake, growth rate and feed efficiency. Douglas *et al.* (1990) using maize, low and high tannin sorghum in broiler diets found that pelleting significantly improved weight gain and feed efficiency regardless of the grain source.

The pancreas weights at 4 weeks of age were in the range 0.32-0.42% of body weight. The pelleted diets gave lower ($P<0.05$) pancreas weights than the mash diets. At 8 weeks of age however, all the diets gave similar ($P>0.05$) pancreas weights ranging between 0.18-0.22%. It is evident that as the

Fig.5. Effect of amaranth level and pelleting

On chick body weight, 1-8 weeks of age



chicks grew and their body weight increased, the pancreas weight expressed as a percentage of the body weight decreased. This demonstrated that various animal tissues developed at different rates as the animal matured. Whereas the trypsin inhibitor content of the mash diets might have influenced the enlargement of the pancreas at the early age, the chicks possibly adapted as they matured and hence overcame any adverse effects.

The pelleting temperature of around 70°C appeared to have been adequate to inactivate some of the antinutritional factors in grain amaranth. The lower pancreas weights given by pelleted diets at 4 weeks of age were evidence of this inactivation.

In related work, Slinger (1973) found that steam pelleting resulted in marked improvement in the nutritional value for chicks of certain feedstuffs such as wheat bran, alfalfa meal, rye and field peas. Most of the improvement was based on enhanced nutrient availability or destruction of heat labile toxins (Slinger, 1973). Similar results were reported by Smits (1993) who studied utilisation of various feeds by pigs.

The level of amaranth had a significant influence on chick body weight at 4 weeks of age but not at 8 weeks. This indicated a higher sensitivity to different diets at the early age compared to older birds. While the young chicks on amaranth diets grew slowly compared to the NRC (1984) standards, the growth rates accelerated as the birds matured

beyond 4 weeks of age. This led to body weights higher than the NRC (1984) standards at 8 weeks of age. The growth patterns in Figure 5 showed increasing growth rate with every successive two week interval for the amaranth diets. However, the highest growth rate for these diets appeared between 4 and 8 weeks of age. For the control diet, the growth rate was faster between two and six weeks of age compared to that of amaranth treatments, but decreased slightly between the sixth and eighth weeks of age. Scott *et al* (1982) similarly showed that the highest chick growth rate was up to six weeks of age followed by a gradual decline beyond this age.

The birds appeared to have adjusted to the higher amaranth inclusion with age thus attaining similar body weights to those on low inclusion diets. This was consistent with the hypothesis that young animals were more sensitive to antinutritional factors than the older ones (Huisman and Tolman, 1992). According to these authors, threshold levels depended on age and species of the animal, in addition to the type of antinutritional factor. A similar development occurred in Experiment 1 whereby chicks on high amaranth inclusion levels had depressed feed intake and growth up to 4 weeks of age but their performance improved significantly in the fifth week of age.

The inclusion of molasses did not ($P>0.05$) affect the body weight, feed intake or feed efficiency. The lack of significant differences in feed intake denoted the failure by molasses to effectively increase the palatability or

acceptability of amaranth diets. In contrast, heat processing was found to increase feed intake possibly through destruction of antinutritional factors (Experiments 3-5). It is evident that steam pelleting also enhanced the acceptability of amaranth diets in the study. Heat treatment thus provided a stronger stimulus for increased feed intake compared to the sweet flavour of molasses in an otherwise unacceptable feed.

The mean mortality rates in this experiment were 5, 6.5 and 11.75% for the control, mash and pelleted diets respectively. Mortalilty was higher after than before the 4th week of age.

Postmortem results showed that most of the deaths were due to ascites. The rapid growth rate of chicks on pelleted diets especially after 4 weeks of age appeared to be related with the high incidence of ascites in those dietary treatments.

8.4.3 Carcass composition

Results of carcass composition at eight weeks of age are presented in Tables 33 and 34. The carcass protein content showed less variation than fat and moisture between the various dietary treatments. The values for the three carcass parameters were in the ranges 15.82-17.17%, 9.76-12.64% and 64.98-69.40% respectively. The pelleted diets gave higher ($P<0.05$) carcass fat levels than the mash diets (Table 34). This trend corresponded with the higher ($P<0.05$) chick body weights of the former diets (Table 32). In addition,

Table 33: Carcass composition and nutrient retention at 8 weeks of age

	20% raw amaranth in diet				40% raw amaranth in diet				Control
	without molasses		with molasses		without molasses		with molasses		Without molasses
Diets	Pelleted 1	Mash 2	Pelleted 3	Mash 4	Pelleted 5	Mash 6	Pelleted 7	Mash 8	Mash 9
Fasted,defeathered, carcass wt.(g)	2062.00	1948.00	2095.00	1982.00	2038.00	1881.00	2045.00	1875.00	2059.00
Moisture,%	64.98^{c}	69.06^{a}	68.27^{ab}	68.86^{ab}	65.69b^{c}	67.72^{ab}	66.50^{abc}	69.40^{a}	68.22^{ab}
Protein, %	16.56^{ab}	16.40^{ab}	16.71^{ab}	17.17^{a}	15.82^{b}	16.08^{ab}	16.41^{ab}	17.06^{a}	16.22^{ab}
Fat, %	12.64^{a}	10.02^{bc}	11.90^{ab}	9.96^{c}	12.53^{a}	10.97^{abc}	11.57^{abc}	9.76^{c}	10.57^{bc}
Ash, %	2.51^{a}	2.64^{a}	2.41^{a}	2.53^{a}	2.24^{a}	2.20^{a}	2.59^{a}	2.59^{a}	2.23^{a}
Protein retention, %	28.80^{a}	29.30^{a}	28.93^{a}	30.10^{a}	26.46^{a}	25.26^{a}	26.79^{a}	27.05^{a}	28.57^{a}
Feather weight, % of body wt.	6.45^{a}	6.47^{a}	6.82^{a}	6.53^{a}	6.59^{a}	6.70^{a}	6.69^{a}	6.50^{a}	6.68^{a}
Carcass nitrogen	2.65	2.62	2.67	2.75	2.53	2.57	2.63	2.73	2.60
Water:Nitrogen ratio	24.52	26.36	25.54	25.07	25.96	26.33	25.33	25.43	26.29

^{ab} Means with the same superscript within a row are not different (P>0.05).

Table 34: Effect of amaranth level, inclusion of molasses and pelleting of diets on carcass composition at 8 weeks of age

	Carcass Moisture	Carcass Protein	Carcass Fat	Carcass Ash	Protein Retention
Mash vs Pelleted	*	NS	*	NS	NS
Molasses vs without molasses	*	NS	*	NS	NS
Level (20 vs 40%)	NS	NS	NS	NS	*

* Significant ($P<0.05$).
NS - Not significant ($P>0.05$).

pelleting significantly reduced ($P<0.05$) the carcass moisture content. However, it had no effect ($P>0.05$) on carcass protein and ash. It is evident that the fast growth rate of chicks on pelleted diets resulted in fatter carcasses.

The generally higher feed intake entailed a higher energy intake. The results were in line with the hypothesis that increases in dietary energy produced heavier but fatter carcasses (McDonald *et al* 1981; Leclerq, 1986; Leenstra, 1989).

Inclusion of molasses resulted in leaner ($P<0.05$) carcasses with a significantly higher ($P<0.05$) moisture content. Replacement of maize with molasses reduced the metabolizable energy content of the diets hence the reduction in fat deposition.

On the whole, the carcass protein levels were slightly lower than the standard value of 18% (Scott *et al*. 1982). However, it should be noted that the carcass composition in the current study was that of the plucked, empty body of the chickens. The plumage removed contained water, ash, lipids and proteins (Emmans and Fisher, 1986). The protein content of feathers is especially high, around 82% (Scott *et al*. 1982). Inclusion of feathers in the carcass composition would hence have increased the protein content.

The carcass composition was similar to that obtained by Keren *et al*. (1990) with 8 week old broiler chickens. These authors however reported slightly higher values for protein and moisture contents and lower ones for the lipid content

since the carcasses analysed had their skin removed. The chicken skin contains considerable levels of fat.

The level of amaranth influenced the retention of protein in the carcass (protein conversion efficiency) (Table 33). Birds on the 20% amaranth level diets had a higher ($P<0.05$) carcass protein retention than those on the 40% level. This reflected more efficient utilization of low amaranth diets with respect to lean tissue growth. This was in conformity with the better ($P<0.05$) feed efficiency of these diets and a generally higher carcass protein.

On the whole, the protein retention values were slightly lower than those reported by Whitehead (1990) for seven week old broilers but within the range reported by Fisher (1980). According to Fisher (1980), the carcass protein growth expressed as the ratio of carcass protein:feed protein was in the range of 0.25-0.35. The low gross protein efficiency values contrasted with the high ones for net amino acid utilisation probably because of the imperfect balance of amino acids in dietary proteins (Fisher, 1980). In the present study, the higher carcass protein: feed protein ratios of birds on 20% amaranth diets compared to those on 40% amaranth indicated a better amino acid balance for protein synthesis with less amaranth in the diet.

Despite the variations in carcass composition between the different diets, the Water:Nitrogen ratio remained almost constant at a mean of 25.65 (Table 34). This was in agreement with values obtained by Summers and Fisher (1961) of 24.2-25.4

for three week old broilers and later reported by Scott *et al.* (1982). Their results indicated that within a single species, the amount of water was closely related to carcass nitrogen over a variety of feeding conditions.

The feather weight at 8 weeks of age ranged between 6.45 and 6.82%. This was close to that of 7% by Scott *et al.* (1982) for 4 week old broilers.

8.4.4 Histopathology

a) Pancreas

On the whole, the birds in this experiment did not show acinar dissociation or necrotic changes in this tissue at both 4 and 8 weeks of age. Generally, there was no lymphocytic infiltration at 4 weeks of age. However, this change was observed in all the treatments at 8 weeks of age except for the control diet. The secretory activity of the acini varied from moderate to maximum but this did not correspond to any pattern of the dietary treatments.

Figure 6 shows a photomicrograph of the pancreas tissue with an area of lymphocytic infiltration.

b) Liver

The changes observed in the chick livers at 4 and 8 weeks of age are shown in Table 35. There were generally more degenerative changes in chicks at 8 weeks than at 4 weeks of age. Similarly, chicks on mash diets exhibited more of these changes compared to those on the control or pelleted diets.

Figure 6: Photomicrograph of pancreas section showing an area of cellular (lymphocytic) infiltration (arrow) . HE X 100

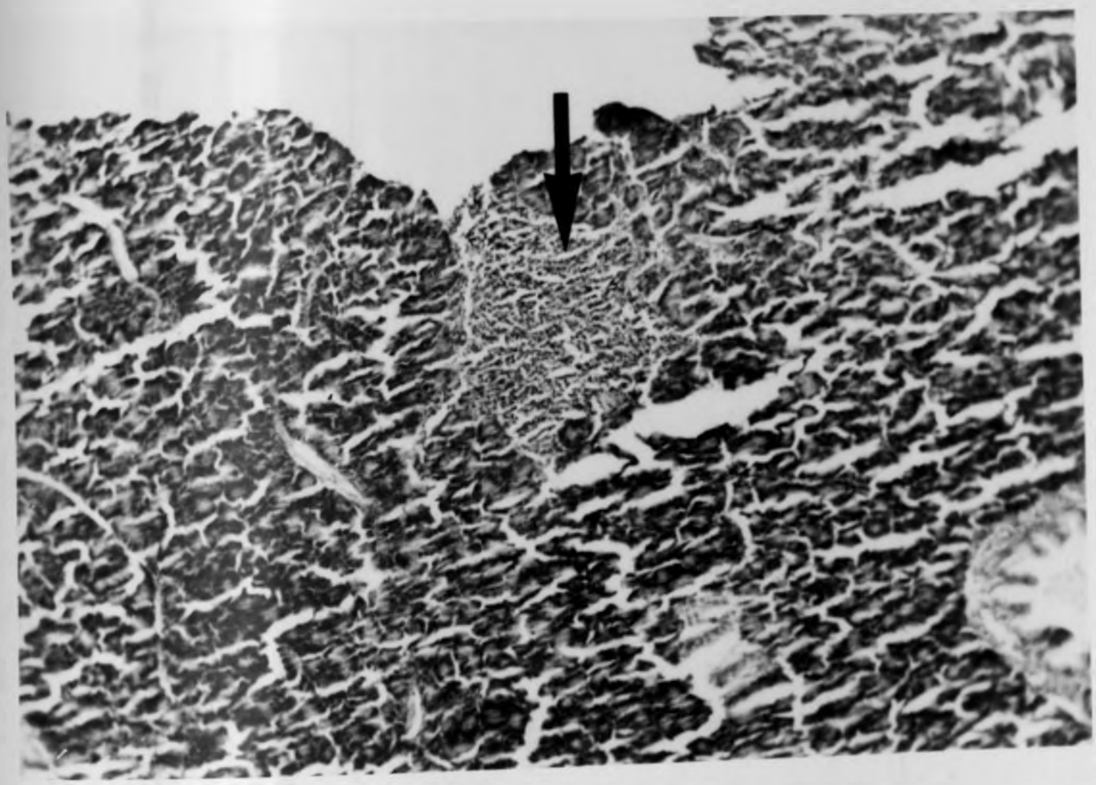


Table 35: Histopathological changes of the liver (Mean score based on degree of severity)

Age of chicks (weeks)	Dietary treatment	Hepatic necrosis	Degenerative changes	Lymphocytic infiltration
4	Mash	-	++	+
	Pellets	-	+	+
8	Control	-	++	+
	Mash	-	+++	++
	Pellets	-	++	++
4	20% amaranth	-	+	+
	40% amaranth	-	++	+
8	0% amaranth (control)	-	++	+
	20% amaranth	-	+++	++
	40% amaranth	-	++	+

Key: - Absent
+ Mild
++ Moderate
+++ Severe

For all the diets, necrosis was virtually absent. Lymphocytic infiltration was higher with the amaranth diets at 8 weeks of age than with the control. Figure 7 is a photomicrograph showing lymphocytic infiltration in a section of the liver tissue.

The differences in histopathological changes of the pancreas and liver between the various dietary treatments were not large enough to be significant. Hence the lack of evidence attributing the changes to the diets.

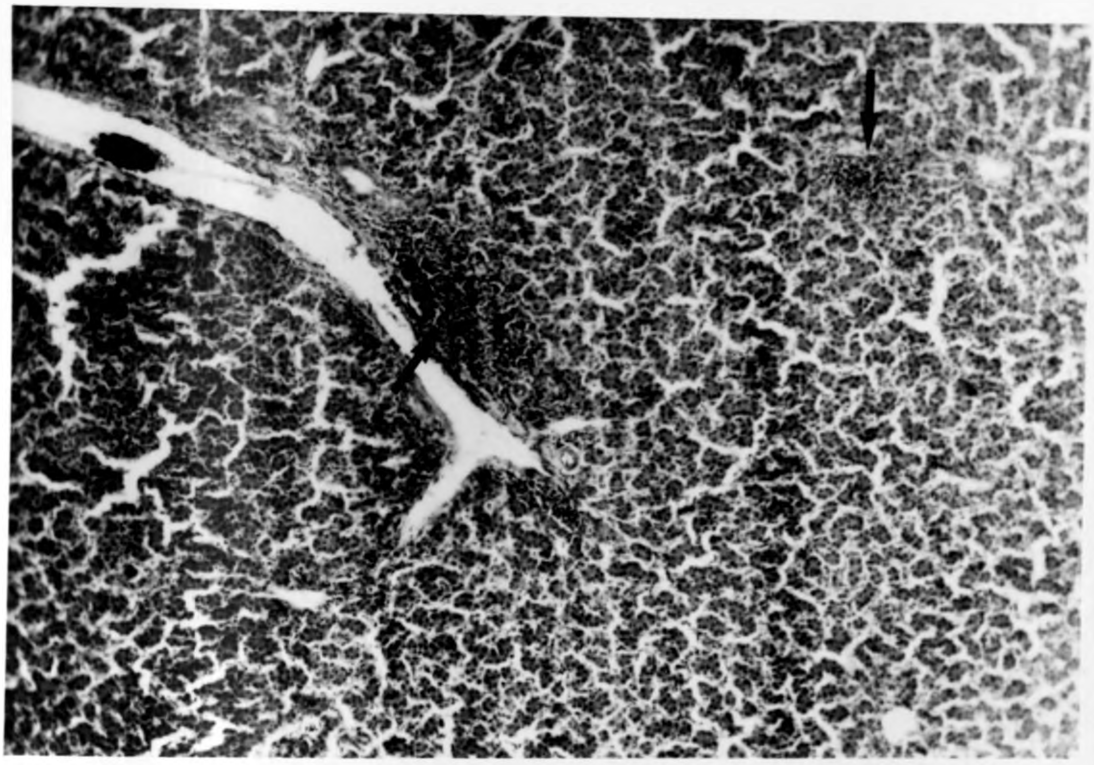
4.5 Mycotoxin examination

The several cases of chicks with ascites in this experiment especially in the pelleted dietary treatments, necessitated the screening of the diets for mycotoxins. The aflatoxins tested were B_1, B_2, G_1 and G_2. Results showed that diet 1 contained minute levels of aflatoxin G_2 at a level of 16.7 ppb. No aflatoxins were detected in the other diets.

The clinical signs of mycotoxicosis are variable and depend on the type of toxin, quantity and duration of intake. Aflatoxins can cause acute poisoning and death in poultry. At the subclinical level, they suppress the immune system, depress feed intake and cause poor growth (Bains, 1979, Robb, 1990). Amongst the toxins produced by Aspergillus flavus, aflatoxin B_1 occurs most often and is the most toxic. Its level of toxicity in chicks is 28.8 mg/kg (Bains, 1979).

Although some of the diets in the present study were pelleted, Bains (1979) noted that mycotoxins were not

Figure 7: Photomicrograph of liver section showing foci of cellular (lymphocytic) infiltration (arrows). HE X 100



destroyed by the pelleting process at the feedmill. However, the levels detected from the screening were negligible and could not be associated with the ascites problem.

8.5 Conclusions

a) Grain amaranth, replacing 20 or 40% of maize resulted in high body weights at 8 weeks of age (no different from the control).

b) Pelleting of amaranth diets resulted in faster chick growth than feeding the diets in mash form.

c) Chicks fed on pelleted diets had fatter carcasses than those on mash diets.

d) Inclusion of molasses had no effect on chick performance.

e) Utilisation of amaranth diets improved as the chicks matured beyond 4 weeks of age.

f) Histopathological changes were generally moderate and could not be attributed to the feeding of amaranth.

g) Birds on 20% amaranth diets had a higher ($p<0.05$) carcass protein conversion efficiency than those on 40%.

CHAPTER 9

9.0 GENERAL DISCUSSION

The potential of grain amaranth as a feed ingredient for poultry has in the recent past led to various nutritional evaluation studies as highlighted in the literature. Most of the work concentrated on the effect of dietary inclusion levels on broiler chicken performance. Some authors concluded that up to 20% raw and 40% heat processed (autoclaved or extruded) grain amaranth could effectively be included in chicken diets (Waldroup *et al.* 1985; Tillman and Waldroup, 1986 and 1988).

Grain amaranth is similar to maize in terms of metabolisable energy. However, it is better than maize in terms of total protein and level of essential amino acids. Based on these attributes only, grain amaranth can replace maize in broiler diets. In this study, the level of tannins and trypsin inhibitor were determined. Although these are negative feed factors, their levels would not severely limit the use of grain amaranth in poultry feeds. The tannin levels were indeed lower than those of sorghums commonly used in poultry feeds (Lorenz and Wright, 1984).

Five feeding trials were carried out in this study (Experiments 1-5). In Experiment 1, inclusion of raw grain amaranth even at a low level (20%) resulted in poor growth rate. Three species of the grain were tested. Only *A.*

hypochondriacus showed similar potential as maize in promoting chick growth when included at 20% of the diet. Again in Experiment 5, raw *A. hypochondriacus* grain at 20% inclusion resulted in similar ($p>0.05$) chick performance as the control diet at 4 weeks of age. By 8 weeks of age, the body weights of chicks were similar ($P>0.05$) between the 0, 20 and 40% raw amaranth level treatments.

Table 36 gives a summary of chick performance where the raw and heat processed amaranth diets were compared to the maize control diets. The data was calculated from the mean weight gain per day and mean feed intake per day in the various trials. The variety *A. hypochondriacus* was selected because it was used in all trials. The table shows that the highest reduction in chick performance came from the raw amaranth diets. The reduction increased as the level of dietary amaranth increased. There are three observations with the raw amaranth diets. Firstly, the reduction in body weight was more severe than that of feed intake. Secondly, the decline in body weight was greater when amaranth inclusion was increased beyond the 20% level than between 0 and 20%. Thirdly, the highest drop in feed intake occurred above the 40% amaranth inclusion level. These results showed that the chick was effectively able to utilise up to 20% dietary amaranth. The severe drop (15.27%) in body weight between 20 and 40% inclusion resulted from a gradual reduction (5.21%) in feed intake. This reflected the increasing rate of decline in feed utilisation as the dietary amaranth level increased.

Table 36: Summary of chick performance; Amaranth diets versus maize control diets

	Amaranth level in diet, %			
	0 (control)	20	40	60
1. Raw A.hypochondriacus diets				
Overall mean weight gain (% of control)	100.00	91.34	76.07	64.57
Overall mean feed intake (% of control)	100.00	95.46	90.25	80.65
2. Processed (extruded) A.hypochondriacus diets				
Overall mean weight gain (% of control)	100.00	99.01	95.69	89.00
Overall mean feed intake (% of control)	100.00	99.01	99.54	97.94
3. Processed (pelleted) A.hypochondriacus diets, (up to 4 weeks of age)				
Overall mean weight gain (% of control)	100.00	92.77	90.18	
Overall mean feed intake (% of control)	100.00	99.15	95.12	

Both the extruded and pelleted amaranth diets showed better weight gain and feed intake than the raw amaranth diets. The extruded amaranth diets showed a drop in chick body weight of only 0.99% between 0 and 20% amaranth inclusion level, 3.32% at 40% level and 6.69% at 60% level. This indicated that the chick was able to utilise these diets efficiently especially up to 40% amaranth level. The reduction in feed intake was negligible up to 40% amaranth inclusion and only 2.06% at 60% inclusion level.

The depression in feed intake observed with raw amaranth diets was attributed to reduced palatability, most likely caused by the presence of antinutritional factors. In addition, the same factors might have interfered with digestibility of these diets. Although the level of tannins was lower than that of sorghum grain, the effect of trypsin inhibitor was found to be substantial in terms of pancreas enlargement. Other suspected ANFs such as saponins and lectins which were not analysed might have contributed to reduced palatability and digestibility of raw amaranth. Inactivation of these factors through heat processing improved the performance of chicks fed on heat processed amaranth diets.

For the pelleted diets, the reduction in broiler performance with increasing amaranth level was not as high as that of raw amaranth diets but was higher than that of the extruded diets (Table 36). It was evident that this type of processing (at 70°C) was not as effective in improving

utilisation of grain amaranth as extrusion (at 150°C). The mild pelleting temperature might not have fully destroyed the antinutritional factors. Koeppe *et al* (1985) reported no loss of trypsin inhibitor activity of *A. hypochondriacus* heated at 70° for 15 minutes. However, the beneficial effect of pelleting in the present study was more striking at the higher level (40%) of amaranth inclusion. The weight gain remained high at 90.18% of that of chicks on the control diet while a similar level of raw amaranth inclusion resulted in only 76.07%.

Table 37 shows the summary of chick performance when amaranth diets were fortified with amino acids, casein, EDTA and molasses in different trials. As in table 36, the data was based on mean weight gain per day and mean feed intake per day for each trial involved. Results of the fortified amaranth diets were then calculated relative to those of the non-fortified ones in each trial.

In Experiment 3, addition of lysine or lysine plus methionine had no effect on chick performance. However, addition of casein improved growth. In Experiment 4, weight gain similarly improved significantly with inclusion of casein. Table 37 further shows that feed intake for the casein diets was higher than that of the non-fortified amaranth diets. Fortifying the diets with EDTA had no effect on weight gain. Inclusion of molasses in Experiment 5 resulted in a slight increase in weight gain (Table 37) which was however not significant (Table 32).

Table 37: Summary of chick performance; Fortified versus non-fortified diets

	Fortification			
Experiment 4 :	None	Lysine	Lysine + Methionine	Casein
Weight gain (% of non-fortified)	100.00	97.30	99.51	104.80
Feed intake (% of non-fortified)	100.00	97.00	98.77	102.50
Experiment 5 :	None	EDTA	Casein	
Weight gain (% of non-fortified)	100.00	99.83	105.46	
Feed intake (% of non-fortified)	100.00	103.94	105.72	
Experiment 6 :	None	Molasses		
Weight gain (% of non-fortified)	100.00	103.24		
Feed intake (% of non-fortified)	100.00	101.78		

Results of this study show that the reduction in weight gain (Table 36) with the raw amaranth diets was largely caused by the reduction in feed intake. However, the severe reduction in weight gain between 20 and 60% amaranth inclusion was in addition to poor feed intake, attributed to a deteriorating rate of feed utilisation. This might have resulted mainly from poor protein digestibility which declined at a faster rate with increase in amaranth level. Poor digestibility of raw amaranth was evident from two observations. Firstly, the mean apparent metabolisable energy of raw amaranth as a percent of gross energy was about 72% (Table 3) compared to that of extruded amaranth of about 77% (Table 13). Secondly, the availability of various essential amino acids was poorer with raw amaranth diets than with the control and processed amaranth diets (Table 21). The amino acid availability indicated the level of protein digestibility. The poor digestibility of raw grain amaranth diets led to the low nitrogen retention observed (Table 20).

The negligible depression in feed intake for the processed amaranth diets up to 60% inclusion showed that grain amaranth in that form was highly acceptable to the chicks (Table 36). This further ruled out the question of an imbalance of amino acids which would have caused poor growth and a considerable depression in feed intake. Chicks on the 60% processed amaranth diet with casein actually gave similar body weight and feed intake as the maize control diet (Table 20). The high nitrogen retention of heat processed amaranth

diets (Table 20 & 25) further affirmed adequate nitrogen utilisation.

The significant growth response from fortification with casein (Table 37) implied deficiency of certain essential amino acids in amaranth diets. Since the addition of lysine and methionine did not increase body weight, it is evident that these two amino acids were not limiting to growth. Casein hence provided some essential amino acids other than lysine and methionine which were limiting. Amino acid analysis of diets used in Experiment 3 (Table 19) showed that casein increased amino acid levels of valine ,threonine, leucine, isoleucine and arginine which were below the NRC (1984) requirements in the amaranth diets. However, the only amino acids amongst these whose levels in the diets were way below those of the maize control diet were leucine followed by threonine. Since the best chick performance came from the contol diet, it may be concluded that the most limiting amino acids in amaranth diets might have been leucine and threonine, in that order. This observation was partly in agreement with that of Becker (1981) who reported that the most limiting amino acid in *A. cruentus* was leucine, followed by valine and threonine.

Chicks on 0, 20 and 40% raw amaranth diets showed differences in body weight at 4 weeks of age but when these diets were continually fed up to 56 days of age, the differences were not significant. Chicks appeared to have adjusted to the higher raw amaranth inclusion diets after four

weeks of age. The birds might have developed some adaptation mechanisms as they matured which enabled them to utilise the diets more effectively. The threshold capacity to cope with effects of ANFs is also known to increase with age (Huisman and Tolman, 1992). In addition, Gous (1986) noted that results from a trial conducted during one stage of growth need not reflect the response at some other stage due to the systematic changes that took place as the bird aged. The improved performance from older birds indicated that grain amaranth might be better utilised in broiler finisher feeds than in starter diets.

In the current study, the level of chick performance for all the diets was generally poorer in the earlier experiments (1 and 2) than in the latter ones (3-5). Certain factors might have played a part in influencing different chick response from similar dietary treatments in different experiments. Amongst such sources of variation, environmental factors might have played a role. The experiments were carried out during different periods of the year. This implied differences in ambient and room temperatures although measures were taken to control the latter. The number of chicks per pen might also have contributed to the chick response. The initial number of chicks per pen in Experiments 1 and 2 was ten while the latter experiments had eight. Ten birds might have created more competition for feed and space despite ensuring that requirements for these two factors were met.

Another factor that varied was the length of storage of grain amaranth. In Experiments 3 and 5, the grain was ground and stored for about two months before using it in the experimental diets. In the other experiments, it was ground and used within the same month. Other factors contributing to different response might have been related to differences in diet composition. The amino acid contents of diets used in Experiments 1, 2 and 5 were not practically analysed. Diets used in Experiment 5 contained higher levels of fish meal (6-8%) than all others which could have contributed to higher levels and availability of amino acids.

Mineral utilisation of chicks on amaranth diets was found to be adequate. Retention of zinc was low and even negative for some of the diets but no deficiency symptoms were observed. The use of EDTA did not enhance mineral availability. The high levels of tissue minerals including zinc indicated efficient utilisation of dietary minerals. The amaranth diets were not different from the maize control diet with respect to zinc availability.

At the cellular level, effects of antinutritional factors on liver, kidney and pancreas were monitored through histopathological examination. Some cases of moderate degenerative and necrotic changes and lymphocytic infiltration were detected. The lack of a pattern consistent with dietary treatments in the various experiments implied that the changes could not be attributed to the feeding of amaranth. No gross pathological lesions were found in these organs.

CHAPTER 10

10.0 CONCLUSIONS

1). Chicks fed on raw grain amaranth diets generally exhibited poor growth compared to those on maize diets. The poor performance was mainly attributed to low feed intake and poor amino acid digestibility.

However, there were no adverse effects on mineral utilisation or on histology of the chicks' internal organs arising from consumption of amaranth diets.

2) Raw *A. hypochondriacus* (1024) grain can effectively replace maize in broiler starter diets at a rate of 20%. This can be increased to 40% if the feed is steam pelleted.

3) Raw *A. hypochondriacus* grain can replace up to 40% of maize in broiler finisher diets, if fish meal (70% protein) is included at a rate above 6%.

4) Extruded *A. hypochondriacus* and *A. cruentus* (434) grain can be included at 40% of the broiler starter diet. A higher rate (up to 60%) can be used if casein is included in the diet at a minimum of 1.5%.

CHAPTER 11

11.0 SCOPE FOR FURTHER WORK

A complete analysis of the antinutritional factors should be carried out to include identification and quantification of lectins, saponins and phytic acid contents in grain amaranth. Inorder to reduce the adverse effects of these factors on animal performance, cheaper methods of processing also need to be investigated. These may include germination of the grain, treatment with saline solutions and various wet cooking methods. The use of higher levels of casein, fish meal and fortification with generally deficient amino acids in amaranth diets such as leucine, isoleucine, threonine and valine require further investigation. The effect of storage period on the nutritive value of the grain should also be determined. Histopathological studies should include examination of the brush border of the small intestines inorder to assess any damage caused by antinutritional factors.

CHAPTER 12

12.0 REFERENCES

Acar, N., P. Vohra, R. Becker, G.D. Hanners, and R.M. Saunders, 1988. Nutritional evaluation of grain amaranth for growing chickens. Poultry Sci. 67: 1166-1173.

Afolabi, A.O., and O.L. Oke, 1981. Preliminary studies on the nutritive value of some cereal-like grains. Nutr. Rep. Int. 24: 389-394.

Arafa, A.S., M.A. Boone, D.M. Janky, M.R. Wilson, R.D. Miles, and R.M. Harms, 1983. Energy restriction as a means of reducing fat pads in broilers. Poultry Sci. 62: 314-320.

AOAC, 1984. Association of Official Analytical Chemists.Official Methods of Analysis - Animal feed section.

Bains, B.S., 1979. A manual of poultry diseases. Ed. 'Roche', Basle, Switzerland. pp. 179-182.

Baker, D.H., 1991. Partitioning of nutrients for growth and other metabolic functions: Efficiency and priority considerations. Poultry Sci. 70: 1797-1805.

Becker, R., E.L. Wheeler, K. Lorenz, A.E. Stafford, O.K. Grosjean, A.A. Betschart, and R.M. Saunders, 1981. A compositional study of grain amaranth. J. Food Sci. 46: 1175-1180.

Betschart, A.A., D.W. Irving, A.D. Shepherd, E.L. Wheeler, and R.M. Saunders, 1980. Nutritional studies on *Amaranthus hypochondriacus* and its milling fractions. In: Proceedings of the Second Amaranth Conference. Rodale Press, Emmaus, PA, U.S.A. pp. 59-60.

Betschart, A.A., D.W. Irving, A.D. Shepherd, and R.M. Saunders, 1981. *Amaranthus cruentus*: Milling characteristics, distribution of nutrients within seed components and the effects of temperature on nutritional quality. J. Food Sci: 46: 1181-1187.

Boorman, K.N., and A.D. Burgess, 1986. Responses to amino acids. In: Nutrient requirements of Poultry and nutritional research. Ed. C. Fisher and K.N. Boorman. Butterworths, U.K. pp. 99-124.

Boorman, K.N., 1992. Protein quality and amino acid utilisation in poultry. In: Recent advances in animal nutrition. Ed. Garnworthy, W. Haresign and D.J.A. Cole. Butterworths, U.K pp. 51-70.

Bressani, R., A. Sanchez-Marroquin, and E. Morales, 1992. Chemical composition of grain amaranth cultivars and effects of processing on their nutritional quality. Food Reviews International 8: 23-49.

Bressani, R., 1988. Amaranth: The nutritive value and potential uses of the grain and by-products. Food and Nutrition Bulletin, Vol. 10; No. 2. The United Nations University. pp.49-59.

Burns, R.E. 1971. Methods for estimation of tannin in grain sorghum. Agron. J. 63: 511-512.

Calderon de la Barca, A.M., J.L. Ochoa and M.E. Valencia, 1985. Effect of the extraction of a hemagglutinin on the nutritive value of Amaranthus leucocarpus seeds. J. Food Sci. 50: 1700-1702.

Carlsson, R., 1980. Quantity and quality of amaranthus grain from plants in temperate, cold and hot subtropical climates - A review. In: Proceedings of the Second Amaranth Conference. Rodale Press, Emmaus, PA, U.S.A. pp.48-57.

Cheeke, P.R. and L.R. Shull, 1985. Natural toxicants in Feeds and Poisonous Plants. AVI Publishing Company Inc., Westport, Connecticut, U.S.A. pp. 33-48; 235-242; 321-322.

Cheeke, P.R., and J. Bronson, 1980. Feeding trials with amaranthus grain, forage and leaf protein concentrates. In: Proceedings of the Second Amaranth Conference. Rodale Press, Emmaus, PA, U.S.A. pp. 5-11.

Chubb, L.G., 1982. Antinutritive factors in animal feedstuffs. In: Recent advances in animal nutrition. Ed. W.Haresign. Butterworths, UK. pp. 21-38.

Dewar, W.A., 1986. Requirements for trace minerals. In: Nutrient requirements of Poultry and nutritional research. Ed. C. Fisher and K.N. Boorman. Butterworths, U.K. pp. 155-172.

Douglas, J.H., T.W. Sullivan, P.L. Bond, F.J. Struwe, J.G. Baier and L.G. Robeson, 1990. Influence of grinding, rolling and pelleting on the nutritional value of grain sorghums and yellow corn for broilers. Poultry Sci. 69: 2150-2156.

Emmans, G.C., and C. Fisher, 1986. Problems in nutritional theory. In: Nutrient requirements of Poultry and nutritional research. Ed. C. Fisher and K.N. Boorman. Butterworths, U.K. pp.9-40

Erbersdobler, H., 1976. Amino acid availability. In: Protein metabolism and nutrition. Ed. D.J.A. Cole, K.N. Boorman, P.J. Buttery, D. Lewis, R.J. Neale and H. Swan, Butterworths. London. pp. 139-158.

Fisher, C., 1980. Protein deposition in poultry. In: Protein deposition in animals. Ed. P.J. Buttery and D.B. Lindsay. Butterworths, U.K. pp. 251-270.

Gadient, M. 1994. New technological aspects in the use of feed additives. Zootecnica International, January 1994, 58-63.

Gous, R.M., 1986. Measurement of response in nutritional experiments. In: Nutrient requirements of Poultry and nutritional research. Ed. C. Fisher and K.N. Boorman. Butterworths, U.K. pp.41-58.

Griffiths, L., S. Leeson, and J.D. Summers, 1977. Fat deposition in broilers: effect of dietary energy to protein balance and early life caloric restriction on productive performance and abdominal fat pad size. Poultry Sci. 56: 638-646.

Gupta, V.K., and D. Thimba, 1992. Grain amaranth: A promising crop for marginal areas of Kenya. Food Reviews International 8: 51-69.

Hadden, G., and Inborr, J., 1993. Stability of dry feed enzymes. Feed Mix 1 (3): 18-23.

Hallmans, G., U. Nilsson, R. Sjostrom, L. Wetter and K. King, 1985. The bioavailability of zinc in endosperm, whole grain and bran-enriched wheat crisp breads fed to rats on a zinc-deficient diet. In: Trace elements in man and animals. Ed. C.F. Mills, I. Bremner and J.K. Chesters. Commonwealth Agricultural Bureaux (CAB), England. pp. 449-450.

Han, I.K., H.W. Hochstetler, and M.L. Scott, 1976. Metabolisable energy values of some poultry feeds determined by various methods and their estimation using metabolisability of the dry matter. Poultry Sci. 55: 1335-1342.

Harris, L.E., and E.W. Crampton, 1973. NRC names for feed processes and their use in evaluating the nutrient content of feeds. In: Effect of processing on the nutritional value of feeds. National Academy of Sciences, Washington, D.C., pp 1-22.

Harper, A.E., N.J. Benevenga, and R.M., Wohlhueter, 1970. Effects of ingestion of disproportionate amounts of amino acids. Physiol. Rev. 50: 423-558.

Herkelman, K.L., G.L. Cromwell, A.H. Cantor, T.S. Stahly and T.W. Pfeiffer, 1993. Effects of heat treatment on the nutritional value of conventional and low trypsin inhibitor soyabeans for chicks. Poultry Sci., 72: 1359-1369.

Hewitt, D., and D. Lewis, 1972a. The effect of dietary lysine level, restriction of food intake and sampling time on the levels of amino acids in the blood plasma of chicks. Br. Poultry Sci. 13: 387-398.

Hewitt, D., and D. Lewis, 1972b. The amino acid requirements of the growing chick. 1. Determination of amino acid requirements. Br. Poultry Sci. 13: 449-463.

Huisman, J. and A.J.M. Jansman, 1991. Dietary effects and some analytical aspects of antinutritional factors in peas, commonbeans and soyabeans in monogastric farm animals. A literature review. Nutrition Abstracts and Rev. 61: 901-921.

Huisman, J., and G.H. Tolman, 1992. Antinutritional factors in the plant proteins of diets for non-ruminants. In: Recent Advances in animal nutrition. Ed. Garnworthy, W. Haresign and D.J.A. Cole, pp. 3-31.

Imeri, A.G., R. Flores, L.G. Elias, and R. Bressani, 1985. Effect of processing on the protein quality of A. caudatus. In: Studies on factors limiting the nutritional quality of raw and processed specific grain amaranth species for food product development. Institute of Nutrition of Central America and Panama. First year report to the National Academy of Sciences, U.S.A.

Jacob, J.P., 1993. The feeding value of Kenyan sorghum, sunflower seed cake and sesame seed cake for poultry. M.sc. Thesis, The University of British Columbia, Canada.

Jain, S.K., K.R. Vaidya and B.D. Joshi, 1977. Collection and evaluation of Indian grain amaranth. In: Proceedings of the first amaranth seminar. Rodale Press, Emmaus, PA., U.S.A.

Janssen, W.M.M.A., 1989. Influence of fibre on digestibility of poultry feeds. In: Recent developments in Poultry nutrition. Ed. D.J.A. Cole and W. Haresign. Butterworths, U.K., pp. 78-93.

Jennings, A.R., 1970. Animal Pathology. Bailliere, Tindall and Cassell, London. pp. 107.

Kakade, M.L., N. Simons, and I.E., Liener, 1969. An evaluation of natural versus synthetic substrates for measuring the antitryptic activity of soyabean samples. Cereal Chem. 46: 518-526

Kakade, M.L., J.J. Rackis, J.E. McGhee, and G. Puski, 1974. Determination of trypsin inhibitor activity of soy products: A collaborative analysis of an improved procedure. Cereal Chem. 51: 376-382.

Kauffman, C.S., 1992. Realising the potential of grain amaranth. Food Reviews International 8: 5-21.

Kenya Bureau of Standards (KBS) 1978. Kenya Standard Specification for compounded poultry feeds. KS 01-61: 1978.

Keren-ZVI, S., I. Nir, Z. Nitsan, and A. Cahaner, 1990. Effect of dietary concentrations of fat and energy on fat deposition in broilers divergently selected for high or low abdominal adipose tissue. Br. Poult. Sci., 31: 507-516.

Koeppe, S.J., J.H. Rupnow, C.E. Walker, and A. Davis 1985. Isolation and heat stability of trypsin inhibitors in amaranth (*Amaranthus hypochondriacus*). J. Food Sci., 50: 1519-1521.

Koeppe, S.J., P.L. Harris, M.A. Hanna, J.H. Rupnow, C.E. Walker and S.L. Cuppett, 1987. Physical properties and some nuritional characteristics of an extrusion product with defatted amaranth seeds and defatted maize gluten meal (80:20 ratio). Cereal Chem. 64: 332-336.

Koeppe, S.J., and J.H. Rupnow, 1988. Purification and characteristics of a lectin from the seeds of amaranth (*Amaranthus cruentus*). J. Food Sci. 53: 1412-1422.

Koide, K., E. Watanabe, M. Toyomizu and T. Ishibashi, 1993. Effect of dietary protein sources on performance and plasma amino acid concentration of broilers. Nutrion Abstracts and Reviews 63(4): 2051.

Laovoravit, N., F.H. Kratzer and R. Becker, 1986. The nutritional value of amaranth for feeding chickens. Poultry Sci. 65: 1365-1370.

Leclercq, B. 1986. Energy requirements of avian species. In: Nutrient requirements of Poultry and nutritional research. Ed. C. Fisher and K.N. Boorman. Butterworths, U.K. pp 125-140

Leenstra, F.R., 1989. Influence of diet and genotype on carcass quality in poultry and their consequences for selection. In: Recent developments in poultry nutrition. Ed. D.J.A. Cole and W. Haresign. Butterworths, U.K., pp. 131-144.

Leibetseder, J., 1980. Introduction to animal olfactory and taste physiology. In: Palatability and flavour use in animal feeds (Advances in animal physiology and animal nutrition journal supplement). First International Symposium, Ed. Hans Bickel, Verlag Paul Parey, Germany. pp. 11-20.

Liener, I.E., 1980. Toxic constituents of plant foodstuffs. 2nd ed. Academic Press, New York, U.S.A. pp. 40-49.

Lipstein, B., 1989. Meat quality in broilers with particular reference to pigmentation. In: Recent developments in poultry nutrition. Ed. D.J.A. Cole and W. Haresign. Butterworths, U.K. pp. 155-156

Lorenz, K., and B. Wright, 1984. Phytate and tannin content of amaranth. Food Chem. 14: 27-34.

Lusas, E. and M. Riaz, 1994. An introduction to extruders and extrusion principles. Extrusion communique, Vol. 7., No. 4 pp 9-16.

Mabray, C.J. and P.W. Waldroup, 1981. The influence of dietary energy and amino acid levels on abdominal fat pad development of the broiler chicken. Poultry Sci., 60: 151-159.

Maga, J.A. 1982. Phytate: Its chemistry, occurence, food interactions, nutritional significance and methods of analysis. J. Agric. Food Chem. 30: 1-9.

Magadi, J.P. 1994. Poultry feed industry; Present and future. Paper presented at the national Poultry Development Programme annual Seminar on 5th July, 1994 by Mr. Magadi of Unga Feeds Ltd., Kenya.

MALDM, 1989. Poultry Production manual, Ministry of Agriculture, Livestock Development and Marketing; Kenya. pp. 70, 71, 78.

MALDM, 1994. Poultry Development Policy. Paper presented at the National Poultry Development Programme annual seminar on 5th July, 1994 by the Deputy Director (Animal Production), Ministry of Agriculture , Livestock Development and Marketing, Kenya.

Makkar, H.P.S., Blummel, M., N.K. Borowy, and K. Becker, 1993. Gravimetric determination of tannins and their correlations with chemical and protein prepitation methods. J. Sci Food Agri 61: 161-165.

Martineau, J.R. 1985. Agronomic summary of grain amaranth. Cereals Research Unit, WRRC-USDA, Albany California.

Maynard, L.A., J.K. Loosli, H.F. Hintz and R.G. Warner, 1979. Animal nutrition. 7th ed. Tata McGraw-Hill Publishing Company. New Delhi. pp. 423.

McDonald, P., R.A. Edwards, and J.F.D. Greenhalgh, 1981. Animal Nutrition. 3rd ed., ELBS, Longman, England.

McNab, J.M., 1980. The concept of amino acid availability in farm animals. In: Recent advances in animal nutrition. Ed. W. Haresign and D. Lewis. Butterworth, U.K. pp. 1-9.

Mendoza, E.G., 1973. Observations of a feed manufacturer. In: Effect of processing on the nutritional value of feeds. National Academy of Sciences, Washington D.C., pp 228-235.

National Academy of Sciences (NAS), 1975. Underexploited tropical plants with promising economic value. Washington, D.C.

National Research Council (NRC) 1984. Amaranth; Modern prospects for an ancient crop. National Academy Press. Washington, D.C.

National Research Council (NRC), 1984. Nutrient requirements of poultry. National Academy of Sciences, Washington, D.C.

Nelson, T.S., L.W. Ferrara, and N.L. Storer, 1968. Phytate phosphorus content of feed ingredients derived from plants. Poultry Sci. 47: 1372.

Oberleas, D. 1985. The effect of phytate on endogenous zinc and zinc homeostasis. In: Trace elements in man and animals. Ed. C.F. Mills, I. Bremner and J.K. Chesters. Commonwealth Agricultural Bureaux (CAB), England pp. 453-455.

O'dell, B.L., J.M. Yohe, and J.E. Savage, 1964. Zinc availability in the chick as affected by phytate, calcium and ethylenediaminetetraacetate. Poultry Sci. 43: 415-419.

Oldfield, J.E., 1973. Effect of fermentation on the chemical and nutritional value of feeds. In: Effect of processing on the nutritional value of feeds; National Academy of Sciences, Washington D.C, pp 228-235.

Pedersen, B., L.S. Kalinowski, and B.O. Eggum, 1987. The nutritive value of amaranth grain (A. caudatus). 1. Protein and minerals of raw and processed grain. Qualitas Plantarum, 36: 309-324.

Peisker, M., 1994. Influence of expansion on compounded feed components. Feed Mix 2 (3): 26-31.

Pharmacia (1988). Handbook amino acid analysis; theory and laboratory techniques. Pharmacia LKB Biotechnology AB, Sweden.

Pharmacia (1988). Instruction manual, LKB amino acid analysis system. Pharmacia LKB Biotechnology AB, Sweden.

Pointillart, A., 1994. Importance of cereal phytases. Feed Mix 2 (3) : 12-15.

Pond, W.G., J.W. Lehmann, R. Elmore, F. Husby, C.C. Calvert, C.W. Newman, B. Lewis, R.L. Harrold and J. Froseth, 1991. Feeding value of raw or heated grain amaranth germplasm. Animal Feed Science and Technology 33: 221-236.

Price, M.L., S. Van Scoyoc, and L.G. Butler, 1978. A critical evaluation of the vanillin reaction as an assay for tannin in sorghum grain. J. Agric. Food Chem. 26: 1214-1218.

Putier, F., 1993. product quality and thermal treatment. Feed Mix 1 (2): 34-37.

Robb, J., 1990. Effects of mycotoxins on animal performance. In: Recent advances in animal nutrition. Ed. W. Haresign and D.J.A. Cole. Butterworths, London. pp. 61-76

Sanchez-Marroquin, A., S. Maya, and L.J. Perez, 1980. Agroindustrial potential of amaranth in Mexico. In: Proceedings of the Second Amaranth Conference. Rodale Press, Emmaus, PA., U.S.A.

SAS Institute, 1985. SAS User's guide: Statistics. SAS Institute Inc., Cary, N.C.

Savage, J.E., J.M. Yohe, E.E. Pickett, and B.L. O'dell, 1964. Zinc metabolism in the growing chick. Tissue concentration and effect of phytate on absorption. Poultry Sci. 43: 421-426.

Sengor, E., and B.E.F. Bayne, 1991. The effects of feed texture on performance in broilers. Nutr. Abstr. Rev. 61(10): 5322.

Senft, J.P., 1980. Protein quality of amaranth grain. In: Proceedings of the Second Amaranth Conference. Rodale Press, Emmaus, PA, U.S.A.

Scott, M.L., M.C. Nesheim, and R.J. Young, 1982. Nutrition of the chicken. 3rd ed., M.L. Scott and Associates, Ithaca, New York.

Shafey, T.M., M.W. McDonald, and R.A.E. Pym, 1990. Effects of dietary calcium, available phosphorus and vitamin D on growth rate, food utilization, plasma and bone constituents and calcium and phosphorus retention of commercial broiler strains. Br. Poult. Sci., 31: 587-602.

Sibbald, I.R. 1976. A bioassay for true metabolisable energy in feeding stuffs. Poultry Sci. 55: 303-308.

Sibbald, I.R., 1986. The T.M.E. system of feed evaluation: methodology, feed composition data and bibliography. Technical Bulletin 1986-4E. Research Branch, Agriculture, Canada.

Sibbald, I.R., 1989. Metabolisable energy evaluation of poultry diets. In: Recent developments in Poultry nutrition. Ed. D.J.A. Cole and W. Haresign. Butterworths, U.K., pp. 12-26.

Simons, P.C.M., 1986. Major minerals in the nutrition of poultry. In: Nutrient requirements of Poultry and nutritional research. Ed. C. Fisher and K.N. Boorman. Butterworths, U.K. pp.141-154

Slinger, S.J., 1973. Effect of pelleting and crumbling methods on the nutritional value of feeds. In: Effect of processing on the nutritional value of feeds. National Academy of Sciences, Washington D.C., pp 48-65.

Smits, B., A.W. Jongbloed and L.B.L. Sebek, 1993. Factors affecting pig diet digestibility. Feed Mix 1 (3): 42-45.

Summers, J.D., and H. Fisher, 1961. Net protein values for the growing chicken as determined by carcass analysis: Exploration method. J. Nutr. 75: 435-442.

Sunde, M.L., 1973. Chemical and nutritional changes in feeds brought about by heat treatment. In: Effect of processing on the nutritional value of feeds. National Academy of Sciences, Washington, D.C., pp 23-33.

Spackman, D.H., W.H. Stein and S. Moore (1958). Automated recording apparatus for use in the chromatography of amino acids. Analyt. Chem. 30: 1190-1206.

Takken, A., and J.K. Connor, 1986. Some toxicological aspects of grain amaranth for pigs. Nutr. Abstr. Rev., 56: 3454.

Teutonico, R.A. and D. Knorr, 1985. Amaranth: Composition, properties and applications of a rediscovered food crop. Food Tech. 39: 49-61.

Tillman, P.B., and P.W. Waldroup, 1986. Processing grain amaranth for use in broiler diets. Poultry Sci. 65: 1960-1964.

Tillman, P.B., and P.W. Waldroup, 1988a. Assessment of extruded grain amaranth as a feed ingredient for broilers. 1. Apparent metabolisable energy values. Poultry Sci. 67: 641-646.

Tillman, P.B., and P.W. Waldroup, 1988b. Assessment of extruded grain amaranth as a feed ingredient for broilers. 2. Apparent amino acid availability values. Poultry Sci. 67: 647-651.

Tillman, P.B., and P.W. Waldroup, 1988c. Performance and yields of broilers fed extruded grain amaranth and grown to market weight. Poultry Sci. 67: 743-749.

Underwood, E.J., 1981. The mineral nutrition of livestock. 2nd ed. Commonwealth Agricultural Bureaux (CAB), England. pp. 135-147.

Van Kempen, G.J.M., 1993. Anti-nutritional factors in animal feed ingredients. Feed Mix 1 (3): 6-9.

Vohra, P., S. Bersch, N. Acar, and R. Becker, 1989. Use of grain amaranth in diets of Japanese quail and chickens. Nutr. Rep. Int. 39: 1227-1235.

Vohra, P., D.B. Chami and E.O. Oyawoye, 1982. Determination of metabolisable energy by a fast method. Poultry Sci. 61: 766-769.

Wallis, I.R., and D. Balnave, 1984. A comparison of amino acid digestibility bioassays for broilers. Br. Poultry Sci. 25: 389-399.

Waldroup, P.W., H.M. Hellwig, D.E. Longer, and C.S. Endres, 1985. The utilisation of grain amaranth by broiler chickens. Poultry Sci. 64: 759-762.

Watkins, K.L. and L.L. Southern, 1993. Effect of dietary sodium zeolite A on zinc utilisation by chick. Poultry Sci. 72: 296-305.

Whitehead, C.C., 1990. Responses of body composition, growth and food efficiency to dietary protein in genetically lean and fat broilers up to 7 weeks of age. Br. Poultr. Sci. 31: 163-172.

Appendix 1: Chemical composition of grain amaranth, maize grain and soyabean meal (air dry basis)

	Grain amaranth[1]	Maize-grain[2]	Soyabean meal[2]
Mean Composition			
Crude protein, %	15.60	8.70	45.00
Metabolisable energy, kcal/kg	3330.00	3370.00	2240.00
Ether extract, %	6.77	3.90	0.90
Crude fibre, %	6.65	2.00	6.00
Calcium, %	0.19	0.02	0.32
Phosphorus, %	0.78	0.30	0.67
Zinc, ppm	26.00	10.00	27.00
Phytate, %	0.57[3]	0.54[3,4]	1.42[5]
Arginine	1.22	0.50	3.20
Cystine	0.33	0.18	0.67
Histidine	0.37	0.20	1.10
Isoleucine	0.50	0.40	2.50
Leucine	0.79	1.10	3.40
Lysine	0.78	0.20	2.90
Methionine	0.32	0.18	0.65
Phenylalanine	0.56	0.50	2.30
Threonine	0.52	0.40	1.80
Tyrosine	0.48	0.41	0.70
Valine	0.56	0.40	2.30

Sources:

[1]Present study - Chapter 3 results

[2]Scott *et al.* (1982)

[3]Lorenz and Wright (1984)

[4]Nelson *et al.* (1968)

[5]Maga (1982)