THE EFFECT OF DUHURRIN PRESENT IN MALTED SORGHUM (Sorghum bicolor L. Moench) ON THE PERFORMANCE OF RABBITS.

BY

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THESIS APPROVAL

This thesis by Suleiman Bogoro meets the regulations governing the degree of Master of Science of Ahmadu Bello University and is approved for its contribution to scientific knowledge and literary presentation.

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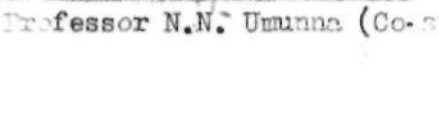
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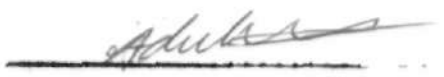


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D E D I C A T I O N

Dedicated to Yellim my wife who dutifully bore the psychological burden while this work lasted.

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GENERAL ABSTRACT

Two experiments were conducted on the University farm, Ahmadu Bello University, Zaria to assess the effect of the cyanogenic glucoside dhurrin present in malted sorghum (sorghum bicolor L. Moench) on the performance of rabbits. Sorghum was malted using Wu and Wall (1980) technique. The first experiment, conducted with weanling (4-week old) rabbits, had two treatments with six replications per treatment. The diets were isonitrogenous and isocaloric. Sorghum inclusion rate was 20%. Feeding lasted for six weeks and records of performance were taken. In the second experiment, rabbits were about 9-10 weeks old. There were three treatments with four replications per treatment. Sorghum inclusion rate was 32% across the diets. Feeding lasted five weeks and growth parameters were recorded. Proximate and cyanide analysis showed that while malting improved the nutritional status of sorghum particularly protein, it alongside raised total and free cyanide contents from 22 to 963 ppm and to 22 to 168 ppm respectively. But even at this level, the rabbits did not show signs of cyanide toxicity. Rather the rabbits on malted sorghum-based diets had better weight gain and feed efficiency. And because bound cyanide which constituted about 83% of total cyanide had to be first hydrolyzed to exert its toxicity, the dangers of the high cyanide levels were already lessened. Malting did not affect feed intake and carcass quality. Generally, rabbits performed better in the first experiment than in the second.

Analysis of physiological indices confirmed previous findings that urine is the major route of cyanide carbon elimination, in form of thiocyanate (SCN^-). Chronic cyanide intake had cummulative effect on body thiocyanate concentration. Removal of roots and shoots from malted sorghum grains depressed performance.

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1. GENERAL INTRODUCTION

In the face of expanding population and diminishing land resources, most countries of the world are forced to lay emphasis on indoor and confined management of animals. For the rabbit which can survive almost exclusively on household food wastes with occasional forage inclusion, keepers find it a convenient animal.

The rabbit has great potential as a meat-producing animal and can produce more meat from forage-based diets than any species of livestock (Cheeke and Paton, 1980). Indeed the rabbit is one of the cheapest producers of meat but hitherto neglected in Nigeria. According to Deblas and Garvey (1975) the rabbit is suitable to raise for meat because of its high fecundity and prolificacy, short generation interval and high feed conversion efficiency. Rabbit meat is nutritionally comparable to that of poultry, and at this time in Nigeria when the high cost of commercial poultry feeds has sent many producers out of the industry the rabbit is a potent alternative considering its ease of management.

Sorghum (Sorghum bicolor L. Moench) ranks fourth among the world's leading cereals. In Nigeria it ranks first and occupies about six million hectares of land. It is one of the most important food and fodder crops of rain-fed agriculture and is widely grown by farmers in tropical Africa especially in the arid and semi-arid subregions due to its partial ability to resist drought conditions. Its agronomic advantages include ease of propagation and lesser fertilizer requirement compared to corn. Along with millet (which is even more drought resistant), they are considered hardy crops (Olufade, 1977).

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Sorghum contains more protein and carbohydrates, less crude fibre and fat than corn (Hulan and Proudfoot, 1983; Okoh et al., 1982). It is also higher than corn in apparent metabolizable energy and all amino acids considered essential for chickens (Hulan and Proudfoot, 1983). Ross and Webster (1960) found that grain sorghum is about 90% as effective as corn for feeding pigs, 95% for feeding beef cattle and 98% as effective for dairy cattle, sheep and poultry production. With pigs, there are palatability factors in the choice of sorghum grain type, near white grain being prefered for feeding to the coloured grains (Loeffel, 1957). Sorghum silage is quite comparable with corn as maintenance ration but needs supplementation with additional grains to compete on a grain basis (Gill, 1971).

Sorghum contains tannin which binds lysine and hence makes lysine a deficient amino acid in sorghum-based diets. This therefore, reduces amino acid availability in high-tannin sorghums (Schang et al., 1986). Tannin is also found to reduce the metabolizable energy of the diets and increase blood cholesterol levels (Vohra et al., 1986).

To increase lysine availability in sorghum, the tannin content of the grains have to be reduced. The techniques that have been employed to reduce tannin content include high-moisture storage or reconstitution (Mitaru et al., 1985) and malting (Wu and Wall, 1980; Kubiczek et al., 1984). Malting has the added advantage of increasing lysine content in addition to

reducing tannin level (Kubiczek et al., 1984). It also aids in the concentration of total sugars due to the conversion of high molecular weight compounds to simpler ones by enzymes during germination (Yakovleve, 1983).

As malting proceeds with reduction in tannin and an increase in amino acids, there is however, a corresponding increase of a cyanogenic glucoside, dhurrin in the sorghum grain. This cyanogenic glucoside, according to Doggett (1970), is a toxic substance which yields hydrocyanic acid (HCN) on hydrolysis in the body of the animal (Okoh et al., 1987). Apparently, the consumption of sorghum sprouts or products made from them may be hazardous because of its high cyanide content. Hydrogen cyanide (also called prussic acid) is a highly toxic substance, 200 ppm being a lethal dose for adults, resulting in death within a few minutes. In small doses, the cyanide is detoxified to thiocyanate by the enzyme rhodanese which makes use of sulphur amino acids as the sulphur donors, thereby making methionine the second limiting amino acid in sorghum-based diets.

From the foregoing, it is evident that previous researches have established that sorghum improves in nutritive status upon malting. But while malting improves its amino acid profile and reduces it tannin content, there is a corresponding increase in its cyanide content. In this light, the purpose of this study is to:

1. assess the response of the rabbits in terms of weight gain and feed efficiency in order to determine if the cyanide formed during the malting of sorghum interfers with the efficient utilization of the improved nutritional attributes in the malted grain;
2. determine the amounts of thiocyanate and cyanide (free and bound) in the gastrointestinal tract (wall and contents), plasma, faeces and urine in order to establish the metabolic fate of dhurrin and the extent of its toxicity to rabbits;
3. see if the removal of roots and shoots from malted sorghum will affect the performance of rabbits feeding on it.

2: LITERATURE REVIEW

THE SORGHUM GRAIN

Sorghum is the genus name for guinea corn, and all cultivated guinea corn races are included in the species bicolor. It is a major cereal crop of the tropics and subtropics. Sorghum ranks fourth among the major cereals (after wheat, triticum eastivum, rice, oryza sativa and corn, zea Mays) in total world production. It is by far the largest cultivated cereal crop in Nigeria (Oyenuga, 1968), occupying about half of Nigeria's total cereal hecterage (Ogunlela et al., 1981). The bulk of the sorghum is produced in the old provincial areas of Kano, Bornu, Adamawa, Bauchi, Katsina, Zaria and Sokoto. Its main ecological advantage is that it does well in areas too dry for corn (maize). Its importance in these areas lies in its being used as a cheap energy source and for various local alcoholic drinks.

Physical and chemical characteristics of the sorghum grain

There are comparatively wide variations in the chemical and nutritive composition of sorghum grain as influenced by the variety and many environmental factors. Trogdon (1960) reported that 67 sorghum samples collected in Texas by the feed control service ranged in protein content from 7.26 to 12.16%. Chemical composition of sorghum as reported by Hubbell (1976) is: protein , 9%, fat, 2.50%; fibre, 2.70%, Ash, 1.80%

dry matter, 89%; calcium, 0.02% and phosphorus, 0.27%. Samford et al. (1970) indicated that all floury types were much spperior to corneous grains with respect to both in vivo and in vitro digestibility. Nishimuta et al. (1969) found that grains containing waxy starch (greater than 75% amylopectin) had higher energy and protein digestibility values.

A major problem encountered in formulating sorghum-based diets for livestock is the large differences in composition among varieties. Ten varieties fed to rats produced significant differences in feed consumed and weight gain (Brever, 1966). Noland et al. (1964) noted differences in performance of swine fed several varieties of sorghum grain. Factors that affect nutritive value of sorghum grain include colour of seedcoat, waxy versus non-waxy starches and moisture content (McGinty, 1972).

But in general, protein content of grain sorghum varies from 6-12% of dry matter (Miller et al., 1964). Many varieties are richer than corn in protein, but lower in soluble carbohydrates, oils and mineral matter. Sorghum protein like other cereal proteins in general, is low in methionine, cystine, tryptophan and somewhat low in histidine. Methionine and lysine are often, however, the limiting amino acids in sorghum-based diets (Elkin et al., 1978). The ash, though somewhat high in phosphorus, is very low in Ca, Na, Cl, Fe, Cu and Co. Feeding tests also seem to

indicate that it is low in vitamin A, and after removing the bran, in members of the B complex vitamins.

Nutritive value

Sorghum grains are a valuable concentrate for man and livestock. The various methods by which sorghum grains may be processed for use in livestock feed include ground, dry-rolled, soaked, pelleted, steam-rolled, extrided, steam-processed, flaked, pressure-cooked, flaked, reconstituted, early-harvested and micronized. Steam-processed, flaked sorghum that is commonly used in large feedlots in the United States increased rate of gain by 7% over dry rolled, 10% over ground grain sorghum with an increase in feed efficiency of 3% and 7% respectively.

The smaller seed varieties and the bran afford excellent food for all classes of livestock. The dried leaves and stems form useful roughage for cattle and horses. The well-matured plant can also be used as green fodder or for silage. As in most other cereals, however, the biological value of sorghum protein is not very high.

METHODS OF TREATING SORGHUM TO IMPROVE ITS NUTRITIONAL QUALITY

Malting

Malting is a process of controlled germination of cereal grain under warm humid conditions so that the ideal biochemical and physical changes can take place. It is a way of improving nutritional value of cereal grains. Malting produces a marked improvement in flavour over that of ungerminated grain

(Novellie, 1976). Good malting cereal usually has white floury or starchy endosperm and low fat which is a desirable trait.

According to Yakovleva (1983), during germination of cereal grain, activation and accumulation of enzymes take place to cause the conversion of high molecular weight compounds into simpler ones and as a result concentration of total sugars in the sprouted grain increases as does the protein quality. Berk (1976) reported that during malting of cereal, the amylolytic activity increases rapidly and that malt contains alpha and beta amylase activity with the whole alpha-amylase produced during germination. During malting the starch in the grain is converted into soluble sugars by the enzyme diastase (Barret and Larkin, 1974).

Germination has been reported (Dalby and Tsai, 1976) to increase the protein content of many cereals like wheat, barley, oats and rye. Kubiczek et al. (1984) reported that total protein percentage of sorghum increased sharply between 6 and 10 days of germination. Wu and Wall (1980) reported that the six-day sprouted normal sorghum had higher protein than the starting material. They also observed an increase in protein and a decrease in starch contents for the 10-day sprouted grain. They observed that the percent protein in germinated sorghum was greater than in the initial grain during germination, but the absolute amount of protein per kernel was not increased.

They concluded that the real nutritive value of sorghum protein after sprouting will have to await human or rat-feeding studies.

Research on local varieties of sorghum in Nigeria have produced similar results. Ikediobi et al. (1987) have reported protein increase in the SK 5912 variety during germination.

In an experiment by Wu and Wall (1980), normal and high-lysine sorghums were germinated for 1-10 days. The lysine content of germinated normal sorghum increased after 10 days from 2.2 to 3.6g/16g of nitrogen. For sprouted, high-lysine sorghum, lysine content increased from 3.0 to 7.8g/16g nitrogen after seven days. A large increase in albumin (rich in lysine) and a large decrease in kafirin and cross-linked kafirin (both low in lysine) accompanied sprouting. In an experiment studying lysine and tryptophan level changes during germination of maize seeds, Tsai et al. (1975) reported that within the first four days of germination of maize at 28°C, the overall levels of lysine and tryptophan increased while there was an accompanying decrease in the amount of nutritionally-poor protein, zein (prolamine). This increase in lysine and decrease in prolamine during germination strongly correlated with the amino acid changes observed by Dupuy et al. (1971) in corn and by Folkes and Yemm (1958) in barley. Lysine content of germinated oat increased after eight days from 3.4 to 5.3g/16g nitrogen (Wu, 1983). Wang and Fields (1978) found that available lysine increased 2.2 times,

methionine 1.8 times and tryptophan 5.7 times for sorghum germinated for 3-4 days at 30°C over the same amino acid values of the control samples based on microbiological assay.

Sorghum grains contains tannin which binds lysine. The presence of tannin in some cultivars of sorghum has been associated with depression of growth rate, feed intake, protein digestibility, metabolizable energy (ME) and also with leg abnormalities in chickens (Chang and Fuller, 1964; Connor et al., 1969; 1975; Herstard, 1979; Elkin et al. 1978). According to Butler (1981) tannin content of 2-3% dry weight sorghum seed is sifficient under optimal conditions to bind considerably more proteins than is present in the seed. Thus dietary tannins may be available to bind proteins of the digestive tract and reduce protein ingestion and absorption. He also found that during germination of sorghum grain, the amount of assayable tannin decreased, which in case of high-tannin lines should mean an improvement in their nutritive value. Kubicmek et al. (1984) observed a rapid decrease (30-70%) of absolute tannin content of grain when two varieties of sorghum namely SK 5912 and SSH 3 were germinated for 4-6 days. Dicher (1975) and Watson and Novellie (1976) reported that the polyphenol (tannin) content of sorghum varieties is reduced by malting. Reichert et al. (1981) reported that germination decreased extractable tannins. Furthermore, weight gains and feed to gain ratios of rat fed germinated sorghum grains were equivalent to those of the rats given sorghum with low-tannin

content. High tannin content of sorghum has been found to lower the protein digestibility of grains in pigs (Ford and Hewitt, 1979). However, reduced digestibility of energy has also been reported in pigs by Noland et al. (1981). Okoh et al. (1982) stated that an appropriate amino acid balance and a low tannin content will generate a better utilization efficiency and also reduce the cost of supplementing sorghum-based rations with limiting essential amino acids.

The production of malted sorghum leads to the emergence of the cyanophoric vegetative portions with its attendant high cyanogenic glucoside (dhurrin) content, which may reach a concentration of 3-5% on dry weight basis within 3 to 4 days (Conn, 1969). Terrasiuk and Bills (1984) found that the average of 613 ppm HCN obtained in the laboratory from germinated sorghum seed, exceeds the average fatal dose ofor an adult. Conn (1969) reported that the young vigorously-growing green leaves of the sorghum plant are a rich cyanide source, the concentration often reaching 0.2% (2,000 ppm) in such leaves. This is why the young sprouts are not normally used as forages, but the more mature dried leaves which contain less cyanide are used for livestock feeding.

Jones (1972) stated that although several animals and plants are able to eat or parasitize cyanogenic plants, this does not detract from the possible basic functions of cyanogenesis as a defence mechanism. No defence is absolute

and so cyanogenesis could be considered only in comparative and not in absolute terms as a defensive mechanism.

Reconstitution

The process of adding sufficient moisture back to grain and sealing the moist grain from the environment so that fermentation can occur has been termed "reconstitution" (Lichtenwalner et al., 1979). Mitaru et al. (1983) undertook a three week experiment with broilers and discovered that reconstitution (25% w/w distilled water added) of high-tannin sorghums decreased their extractable tannin contents. Chickens fed diets containing these treated sorghums had improved weight gains (23 to 83%) and feed efficiencies compared to birds fed diets containing the untreated grain. Treatment of low-tannin sorghum did not, however, improve weight gains. Reconstitution of high but not low-tannin sorghums improved protein digestibility (6 to 16%) and dietary metabolizable energy (0.1 to 0.3 Kcal/g) for broiler chickens. Reichert et al. (1980) showed that anaerobic storage of moist grain deactivated tannins and improved the nutritional quality of the high-tannin sorghum for rats.

Fermentation

Grain sorghum silage (made in the manner of any standard silage) can be fed to livestock (McGinty, 1972). Results of trials over three yields by Pund (1970) showed distinct quality difference between a bird-resistant and a non-resistant

grain sorghum. Silage with the latter hybrids was superior in all traits from daily gain and feed efficiency or through dressing percentage, marbling and carcass grade. Sorghum silage is quite comparable with maize as maintenance ration but needs supplementation with additional grains to compete on a grain basis (Gill, 1971). Doggett (1970) has also reported that ensiling completely destroys hydrocyanic acid in sorghum.

CYANIDE IN SORGHUM AND OTHER FOODSTUFFS

About 1000 plant species representing 90 families and at least 250 genera have been reported to be cyanogenic (Dilleman, 1958; Hegnauer, 1963). Several dozen species have been studied in greater detail, in some instances because of their economic significance, and 12 cyanogenic glycosides have been identified (Conn, 1969). The majority of these are formed by the plant from one of four amino acids (valine, isoleucine, phenylalanine and tyrosine) by means of a biosynthetic pathway involving oximes, nutriles and x-hydroxynitriles (Cyanohydrins).

The ability of plants to produce cyanide from a parent compound was first reported when Schrader (1803), a German pharmacist, recognized the odour of hydrocyanic acid produced by the cherry laurel and other plants (Oyenuga, 1968). The presence of hydrocyanic in bitter cassava roots was first reported by Boutron-Charland in 1836. Francis (1878) showed that the acid is also yielded by the roots of the sweet cassava

variety (Oyenuga, 1967). Cyanogenic glucosides are toxic because they yield hydrogen cyanide (HCN) when enzymatically-degraded. Enzymatic hydrolysis results when the plant tissue is crushed or otherwise disrupted, but hydrolysis by the digestive enzymes of an animal feeding on cyanophoric plants may also occur.

Cyanogenic glucosides (or glycosides) are compounds which liberate HCN, one or more molecules of sugar, and with one exception (Nimington, 1935), an aldehyde or ketone on treatment with dilute acid or the appropriate hydrolytic enzymes. These compounds have a wide distribution among the higher plants (Dillemon, 1958) but are also found in insects (Blum and Woodring, 1962; Eisner et al., 1963; Jones, 1962) and fungi (Tschiesch, 1967). Structures of known cyanogenic glycosides are described in Table 1.

Amygdalin which is probably the most familiar of the cyanogenic glycosides and is found in many members of the Rosaceae, is a β-glycoside of D(-) Mandelonitrile or benzaldehyde cyanohydrin. On hydrolysis, this glycoside yields two moles of glucose and one mole each of benzaldehyde and HCN. An enzyme in snails (Giaja, 1919) acts on amygdalin to cleave the linkage between the cyanohydrin and sugar moietes and form gentiobiose. Amygdalin was the subject of many chemical studies during the 19th century (Robinson, 1930). Its structure was finally considered established when Campbell and Haworth (1924) synthesized the cyanogen.

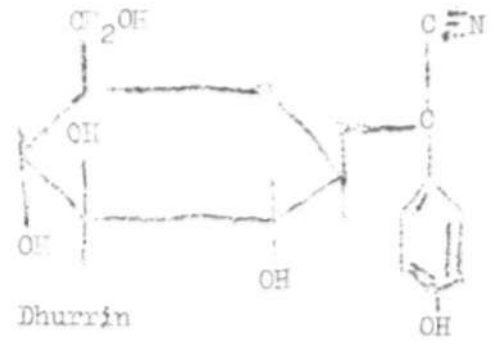
Table 1 : The twelve known cynnogenic glucosides (Dillemen, 1958).

Glycoside	Sugar	Aglycone	Occurence
Amygdalin	Gentiobiose	D-mandelonitrile	Prunus spp.
Prunasin	D-Glucose	"	Prunus spp., Many Rosaceae, Eucalyptus spp.
Sambunigrin	"	"	Sambucos nigra, Acacia spp. (Australin)
Prulaurasin	"	D-mandelonitrile	
Vicianin	Vicianose	D-mandelonitrile	Vicia angustifolia L. and other vicia
Dhurrin	D-Glucose	L-P-Hydroxymandelonitrile	Sorghum spp.
Taxiphyllin	"	D-P-Hydroxymandelonitrile	Taxus spp.
Linamarin	"	-hydroxyisobutyronitrile (acetone cyanolhydrin)	Manihot spp., Phaseolus Lunatus, L.; Linum Usitatissium, L.; Trifolium repens, L.; Lotus spp.
Lotaustralin	"	-hydroxy-&-methyl Butyronitrile (methlethyl ketone cyanohydrin)	(See Linamrin)
Zierin	"	m-Hydroxymandelonitrile	Zieria laevigata sm.
Acacipetalin	"	-Dimethyl-&-Hydroxylonitrile	Acacia spp. (South African)
Gynocardin	"	Gynocardinonitrile	Gynocardia odorate, R. Br. Panguim edule, Reinw.

Many of the glycosides contain mandelonitrile as the aglycon. The asymmetric carbon atom at the point of attachment of the glucose provides the possibility of stereoisomerism, and the diesteroemer of prunasin containing L(+) mandelonitrile is known as sambunigrin after the plant sambucos nigra L., in which it was first detected (Bourquelot and Danjou, 1905; Guignard, 1908). An equimolar mixture of the two compounds known as prulaurasin (Herrissey, 1906) is related to amygadalin in that the sugar component is dissacharide vicianose, consisting of one molecule.

The sorghum genera contains the glucoside dhurrin, the aglycone of which is p-hydroxymandelonitrile (Dunstan and Henry, 1902). A positional isomer of dhurrin is the compound Zierin which is found in an Australian rutaceous shrub, Ziera laevigata s. (Finnemore and Cooper, 1936).

Still another isomer of dhurrin is p-glucosyloxymandelonitrile, the labile cyanogenic glucoside of



Dhurrin

Nandina domestica Thunb., the ornamental shrub known as heavenly bamboo (Abrol et al., 1960). In this compound, the glucose is attached to the aromatic hydroxyl, and the compound is unstable, dissociating to form p-glucosyloxybenzaldehyde and HCN.

Two other cyanogenic glucosides which are widely - distributed (Butler, 1965) in the higher plant Kingdom are linamarin and

lotaustralin, the glucocides of acetone cyanohydrin and methylethlketone cyanohydrin. An interesting aspect of these compounds is that they usually co-exist in the same plant, although they may be present in widely-differing amounts. Thus, the linen flax plant contains a 50-50 mixture of linamarin and lotaustralin while lotus arenarius Brot contains the two compounds in a ratio of 99 to 1. On the other hand, the ratio of linamarin - lotaustralin in lotus tenuis L. is 4 to 96. In tapioca, the ratio is 93 to 7 (Nartey, 1973). Linamarin and Lotaustralin might have been originally identified from other plants, but it appears its significance in contemporary knowledge is almost directly linked with cassava (manihot spp.) research . Although the cyanogenic glucosides are distributed throughout the tapioca plant, the quantities vary greatly between cultivars and the sweet variety are generally of lower level than the bitter one (Coursey, 1973). The normal range of cyanogens is between 15 and 400 ppm, calculated as my HCN per kg fresh weight, but occasionally as low as 10mg per kg or over 2,000mg per pg. Most commonly, it falls between 30 and 150mg per kg (Coursey, 1973). Whole roots of bitter Nigerian cassava averaged 74.5 ppm HCN, whereas those of sweet cassava averaged 32.9 ppm (Tewe, 1983). According to Muller et al. (1974), cyanides are the most unstable constituents of the cassava plant. The content in fresh tubers varies from about 0.01 to 0.04 percent, with the bitter varieties containing 0.02 to 0.03 percent, and the sweet less than 0.01

percent. Free HCN is liberated from the cyanogenic glucosides by the action of the enzyme, linamarase, which is found in the plant. However, the glucosides and linamarase come into contact only when the plant tissue is damanged in storage, or during processing and handling like sun-drying, bailing, ensiling, fermentation e.t.c. Cassava peels have also been reported (Larsen and Amaning - Kwarteng, 1976) to contain HCN in the following proportions: Fresh cassava peels, 156.82 mg/kg; sun-dried cassava peels, 23.98 mg/kg and peel silage, 73.80 mg/kg.

The level of these glucosides in tubers is influenced by environmental conditions, nitrogen fertilization increases while potassium and farmyard manure decrease the level (Yeoh and Chew, 1974). Other factors like genetics, water, lighting e.t.c. which tend to affect cyanogenesis in the cassava plant have prompted increased breeding research by IITA (International Institute for Tropical Agriculture) etc. for the possible discovery of low or zero cyanogenic glucoside varieties (Cock, 1973; Sadik and Hahn, 1973). Finally, it has been proved that sun-drying and heat treatment or preferably a combination of both virtually eliminates HCN (Normanha, 1956; Larseng and Amaning-Kwarteng, 1976; Oke, 1984). Fierce heating which destroys enzyme action must be avoided (Grace, 1971). Investigations into the addition and recovery of cyanide (KCN) and the cyanide retention properties of various types of chicken feed have revealed that cyanide was very unstable in feed. Over 90% could be lost in 24 hours under experimental conditions (Carew, 1981). Practical feeds (composed of natural ingredients) were especially poor in retaining cyanide, compared with semi-synthetic diets.

Biosynthesis of cyanogenic glycosides:

Research in several laboratories have shown that four of the known cyanogenic glycosides are derived from closely-related amino acids (Fig. 1).

Thus tyrosine is known to be effectively converted into dhurrin in the sorghum plant (Conn and Akazawa, 1958; Gander, 1958) and similarly, tyrosine is converted into taxiphyllin in shoots of the yew tree (Bleichert et al., 1966). Phenylalanine has been shown to be converted to prunasin in cherry laurel leaves (Mentzer and Favre-Bonvin, 1961) and in young peach seedlings (Ben-Yehoshua and Conn, 1964). Butler and Butler (1960) first showed the conversion valine to linamarin in New Zealand colver as well as the conversion of isoleucine to lotaustralin. In each of these biosyntheses, there is a loss of the carboxyl carbon and an oxidation of the amino carbon and nitrogen atoms to the levels of a nitrile. Using tyrosine doubly labelled with ^{14}C in the alpha carbon and ^{15}N in the amino group, Uribe and Conn (1966) showed that the nitrile group of dhurrin was derived directly from the precursor amino acid.

These biosynthetic processes are illustrated with linamarin and lotaustralin (Figure 2).

Amino Acids as Precursors of Cyanogens

Fig 1 : Amino acid precursors of four cyanogenic glucosides (Conn, 1969).

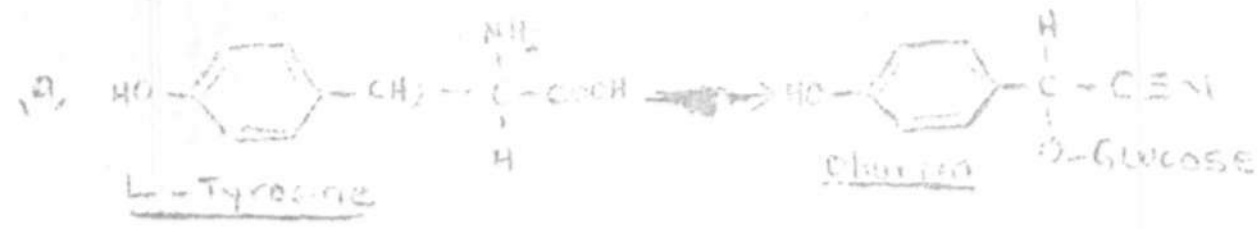
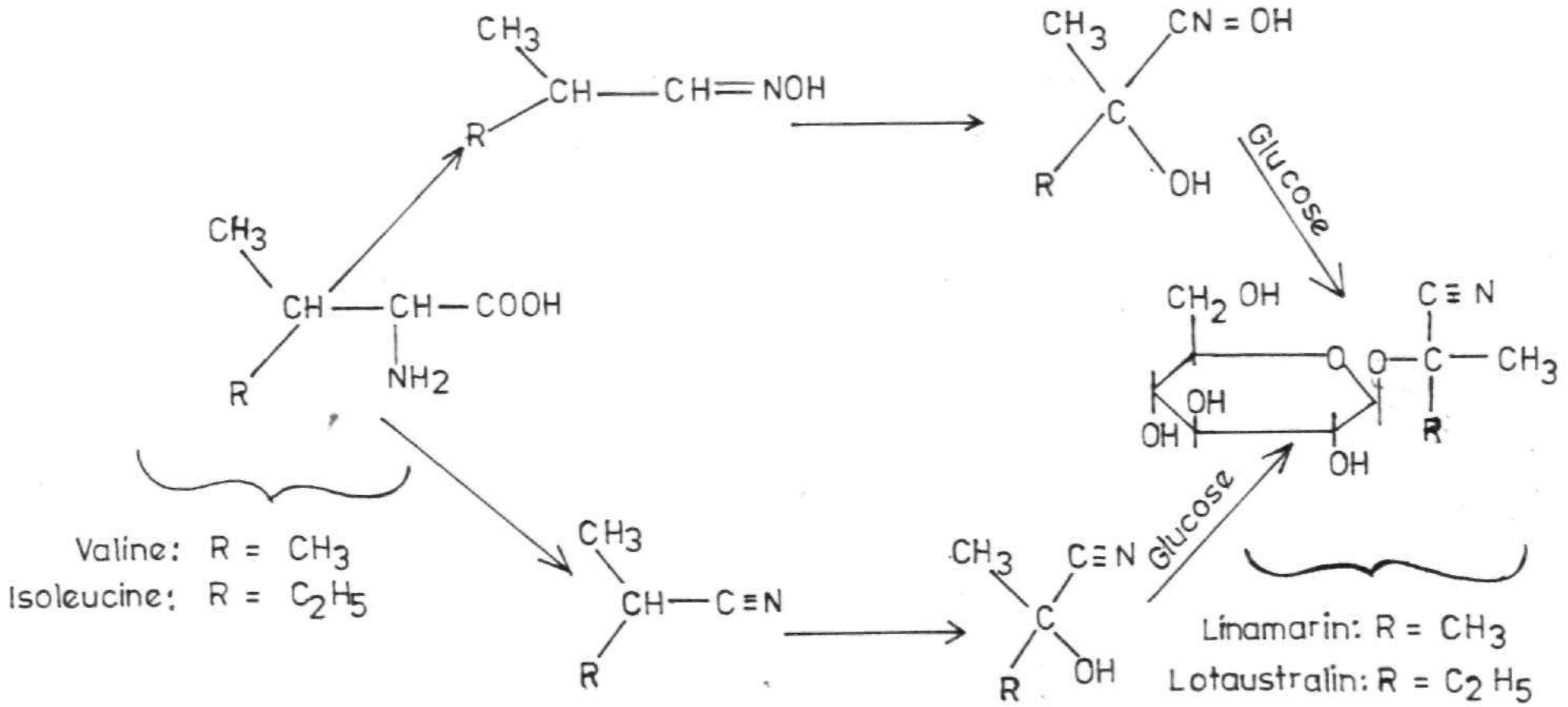


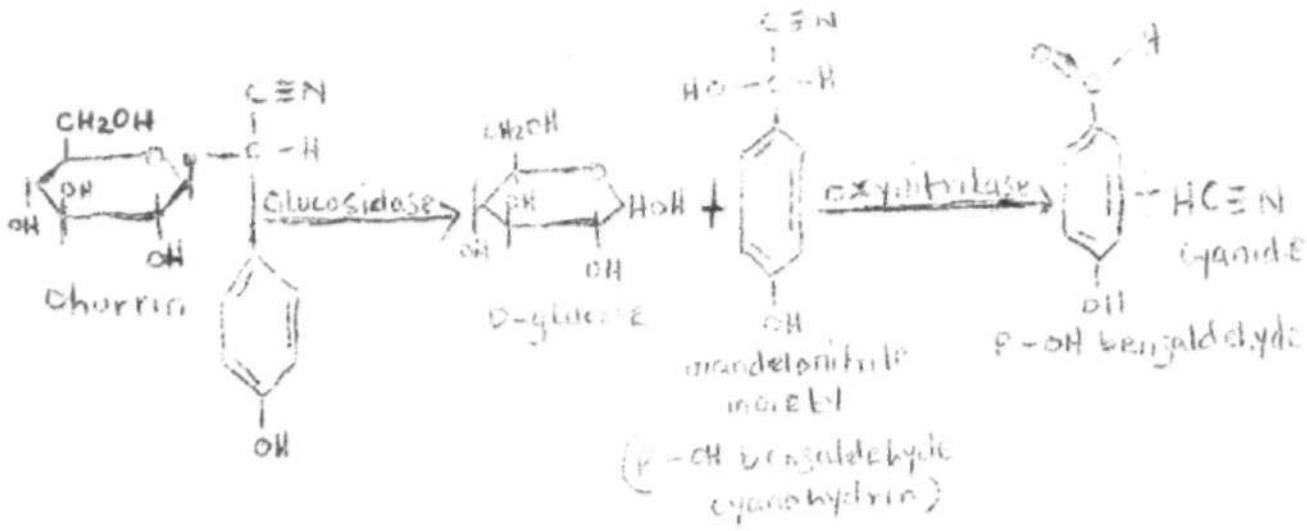
Figure 2: The biosynthesis of linamarin and lotaustralin in tapioca (Nartey, 1973)



Enzymatic degradation of cyanogenic glycosides

Structurally, the aglycone of dhurrin is p-hydroxymandelonitrile. The sugar portion is D-glucose. Dunstan and Henry (1902) demonstrated an enzyme that hydrolyses the glycosidic linkage between glucose and p-hydroxybenzaldehyde cyanohydrin (p-hydroxymandelonitrile) as illustrated below.

Figure 3 : Dhurrin hydrolysis



Ma'o and Anderson (1967) have also reported the isolation of two B-D-glucopyranosidases in sorghum vegetative tissues and seeds, but only one hydrolyzes dhurrin. Cyanogenic glucoside of sorghum has also been investigated by Akazawa et al. (1960). They extracted twenty 3-day old etiolated seedlings (1.39 gm) with ethanol and aerated the ethanoic supernatant to remove and measure HCN. They found 21.0 u moles of HCN per grem of seedlings, a value which

corresponds to the glycoside content of seedlings of this age, and indicates that no hydrolysis of p-hydroxymandelonitrile -B-D-glucoside occured uptill emulsion was added to the residue of the ethanol extract. The absence of HCN in the ethanol extract shows that little or no free HCN exists in the plant.

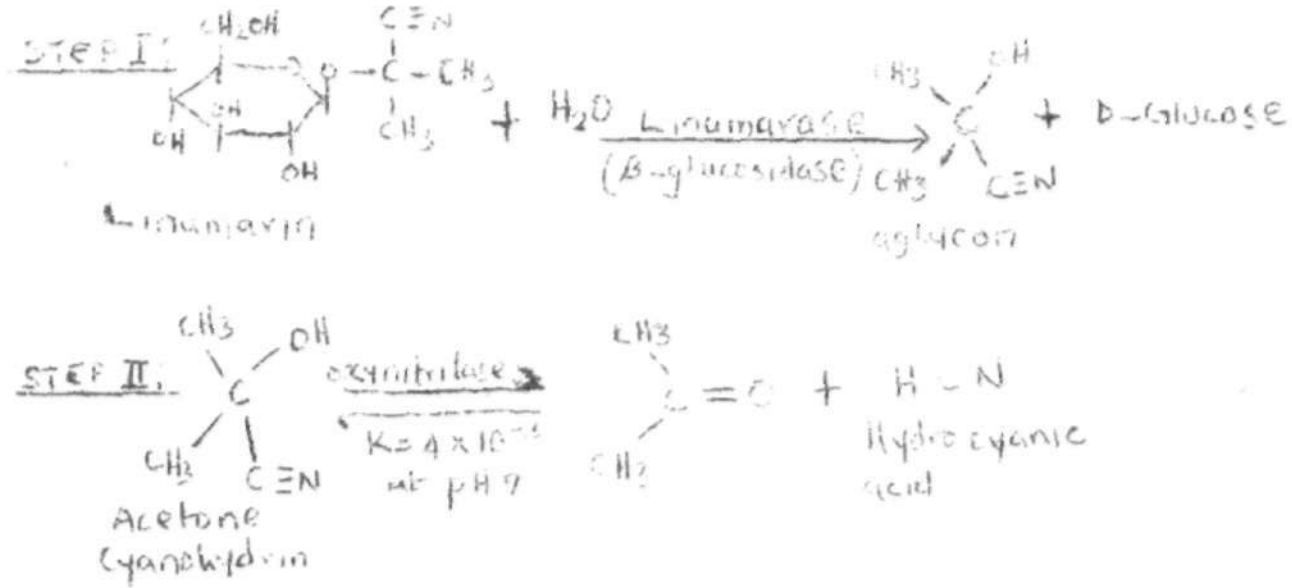
Analysis of aqueous extracts from dry ungerminated sorghum seeds showed neither the presence of hydrocyanic acid nor p-hydroxybenzaldehyde. Since the seed, conceivably might lack the B-D-glucosidase necessary for hydrolysis of the glucoside, 50g of emulsion was added to the aqueous extracts. Neither aldehyde nor hydrocyanic acid was also produced under these conditions. Subsequent digestion of the extract with H_2SO_4 at 80°C for 5 hours also failed to release hydrocyanic acid or the aldehyde (Akazawa et al., 1960).

The localization of the glycoside within the young plant was determined. After digestion and aeration for 16 hours, the shoots contained 18.5 u moles HCN/g. The roots and seed residues contained no detectable HCN and, although these tissues conceivably could lack the glycosidase necessary for enzymatic hydrolysis of the glycoside, hydrolysis with 1.4N H_2SO_4 for 5 hours at 80°C failed to produce any hydrocyanic acid. By contrast, the work of Akazawa et al. (1960) show that at room temperature, alkaline treatment results in hydrolysis of the glycosidic bond to yield p-hydroxymandelonitrile which in turn decomposes to p-hydroxybenzaldehyde and HCN. However when amygdalin was treated

with 0.1 N NaOH under the same conditions, hydrolysis was not achieved. This is in agreement with the work of Ballou (1954) who showed that only certain glycosides are alkaline-labile.

The crude preparation of enzymes from almonds, knowns as emulsion has long been recognized to carry out the complete degradation of amydalin and other glycosides into sugar, hydrocyanic acid and an aldehyde or ketone. The degradation process may be broken down into two separate enzymatic steps as illustrated for the cyanogen linamarin (Figure 4).

Figure 4 : The enzymatic degradation of Linamarin (Conn, 1969)



Only recently have the cyanogenic glucosides been purified and studied. Mato and Anderson (1967) have purified the B-glucosidase in sorghum which catalyzes the hydrolysis of dhurrin and found that it has rather broad specificity with respect to its substrate.

METABOLISM OF CYANIDE IN ANIMALS

Following injection of Na ^{14}CN in dogs (Boxer and Rickards, 1952), ^{14}C appeared in urine, in free cyanide as a constituent of cyanocobalamin and in thiocyanate. A part of the cyanide and thiocyanate carbon is oxidized directly to form CO_2, which is achieved, according to Boxer and Rickards (1952), probably via cyanate. The carbon of cyanide also appeared in the carbon of the methyl groups of choline and methionine and in the urea carbon of allantoin as well. The formic acid isolated in these experiments contained a high amount of ^{14}C. Based on this fact, formic acid was considered as a starting material for the introduction of the cyanide carbon into one-carbon metabolism (Boxer and Rickards, 1952).

In another radioactive tracer experiment, Okoh and Pitt (1982) showed that rats injected with Na ^{14}CN converted the bulk of the cyanide into thiocyanate. The thiocyanate formed in the tissues of the test animals was shown to be secreted into the stomach and reabsorbed in the lower part of the gut into the body fluid to be partly excreted in the urine and partly resecreted into the gastric juice. Thus in the rat, there is a gastrointestinal circulation of thiocyanate linked with the thiocyanate pool (Okoh and Pitt, 1982). The major metabolite of cyanide in animals is thiocyanate (Boxer and Rickards, 1952; Okoh , 1983) and this is excreted largely in the urine (Crawley and Goddard, 1977; Okoh, 1983).

CYANIDE TOXICITY AND DETOXIFICATION

Cyanide toxicity

The cyanide content of animal forage crops such as sorghum, New Zealand clover, and lima beans used as cattle feed has frequently been responsible for livestock poisoning. Several ornamental plants - heavenly bamboo, Nandina domestica, and the ornamental Yew (taxus species) are cyanophoric. The concentration in cherry laurel leaves may run as high as 75 u moles gm fresh weight corresponding to about 1% hydrocyanic acid on a dry weight basis. Infact squashed leaves are commonly used by collectors to kill butterflies and moths. The berries of Nandina domestica can contain up to 150 u moles per gm. Obviously, if any significant quantity of these materials is consumed by a human, particularly a child, rapid poisoning will occur. Fortunately, the plants mentioned usually have other bitter components which prevent their being consumed in any large amount (Conn, 1969).

If the concentration of glycoside in a cyanophoric plant is sufficiently high, the plant will be toxic to animals owing to the production of hydrocyanic acid when the plant is ingested. Hydrocyanic acid is a highly-toxic material, 200mg being a lethal dose for an adult human; an atmosphere containing 200 ppm will result in death within a few minutes. In the cell, HCN is an effective inhibitor of many metalloenzymes, but cytochrome oxidase the terminal oxidase of aerobic organisms, is the primary site of action. Cyanide poisoning, therefore, results in death due to

oxygen starvation at the cellular level (Conn, 1969). Based on Bolhuis' (1952) classification of hydrocyanic acid toxicity levels in root-cassava, the 156.8mg/kg content of the fresh cassava peels (Larseng and Amoning-Kwarteng, 1976) makes it too toxic for livestock. However, the levels in both the dried mixture and the peel silage (21.60 and 73.8mg/kg respectively) were non toxic on the same classification.

Common manifestations of cyanide toxicity

Experiments conducted at Trichur (Anon, 1975) have shown that tapioca leaf meal contains about 7.6mg of hydrocyanic acid per 100gm of material. At an intake level of 0.5 to 0.8 percent of the body weight, it does not bring about any adverse effect (Ranjhan, 1980). In another experiment by Oyelola et al. (1983), samples of maternal blood, amniotic fluid, breast milk and urine were taken from 150 pregnant women of different socio-economic status having been fed cassava diets. The samples were analyzed for thiocyanate. The mean thiocyanate level was higher in the plasma (20.52m mole/l) with a range of 0-83.00m mole, followed by the urine (18.9 m mole) with a range of 3.10-37.60m mole. Although the range was smaller in the amniotic fluid than the foetal plasma (6.78 mole). Only the maternal plasma thiocyanate level gave a significantly positive correlation with the foetal plasma ($P<0.05$). All the other correlations were insignificant. The content in the breast milk (16.81M mole) may be sufficiently high enough to affect the thyroid function of a breast-fed infant in the presence of insufficient iodine and this may possibly be

the etiology of endemic goitre. A related situation is the finding by Carew (1981) that the major adverse symptoms exhibited by chicks chronically exposed to large amounts of thiocyanate was thyroid enlargement. Also, cassava intake has been suspected by several local clinicians in South East Nigeria as a cause of congenital anormalies if consumed in excess during pregnancy. This, however, remains a controversial issue, and precise scientific data is lacking (Sing, 1981). Nartey (1981) enumerates the diseases caused by cyanogenesis in cassava as endemic goitre, depletion of the sulphate pool of the body, vitamin B_{12} deficiency, cretinism (a form of deficiency caused by hypothyrodism seldom accompanied by mental deficiency) and mental retardation, ataxic neuropathy, and in the worst cases, death. According to Singh (1981), reports are on record showing correlation between cassava intake and the occurence of various types of neuropathies. This has previously been well established (Rose et al. 1954; Williams and Osuntokun, 1969; Osuntokun, 1973).

Differences in species response may be significant. Unfortunately, the most detailed research in relation to dietary cyanide content has been carried out with small laboratory animals, and the results of this work may be of limited relevance to farm livestock (Nestel and Graham, 1977).

Detoxification of cyanide

Detoxification is a broad term that denotes any method or process whereby cyanide is removed from its precurssor (the glycoside) in cyanogens or in bound forms in the body in any convenient way

as to limit its toxicity in the body of animals. The first type is essentially a physical process or a chemical process initiated by physical measures like soaking, boiling etc.

Narashima (1933) found that drying side branches and young tillers of sorghum in the shade decreased only about 10% of cyanide; sundrying showed only partial decrease (30-40%) while heating tissues to 100°C and maintaining at that temperature for some hours destroyed the poisonous principle. As earlier reported (Doggett, 1970), ensiling completely destroys hydrocyanic acid in sorghum.

Traditional detoxification methods for cassava and cassava-based foods include the following (Coursey, 1973):

a. cooking techniques which include boiling, roasting and frying
b. sundrying, with or without processing like milling, grinding etc.
c. kiln or hot-air drying
d. detoxification by solution which includes soaking and boiling.
e. detoxification by fermentation which could be followed by washing and heat-treatment like roasting, steaming and drying in hot air.

If linamarin is itself non toxic, presumably all that is necessary would be heating sufficient to denature the hydrolytic enzyme and so prevent the release of free hydrocyanic acid. In practice, however, most tradiational food preparation techniques appear to be designed to bring together enzyme and substrate by cell rapture, followed by elimination of the liberated hydrocyanic

acid by either volatilization or solution in water. In some processes, the initial hydrolysis of the linamarin is assisted by the use of fermentation processes (Coursey, 1973).

The pathways for the detoxification of cyanide have been reviewed by Oke (1973). The major detoxification product of cyanide is thiocyanate. The high amount of thiocyanate found in the urine, saliva and blood of people who eat a lot of cassava is from the detoxification of the cyanide. There are two possible pathways for the formation of thiocyanate as illustrated in Figure 5. In one pathway, L-cysteine, which serves as the sulphur donor is transaminated to 3-mercaptopyruvate and the sulphur in turn is transfered to a sulphite radical by sulphur transferase, forming thiosulphate and pyruvic acid in the process. Rhodanese catalyzes the reaction between thiosulphate and cyanide to produce thiocyanate and sulphite. The sulphate combines with sulphur (catalyzed by sulphur transferase) to produce S_2O_3 thereby entering the cycle again. The pyruvate produced goes into the TCA (tricarboxylic acid) cycle while thiocyanate is excreted.

Methionine can also provide sulphur for the rhodanese system by conversion to cysteine (Figure 6). This important role by methionine has been recognized for long (Oke, 1973). Improvement in poultry performance following methionine supplementation of cyanide-based diets have been reported by Carew (1981) and Ngoka (1986).

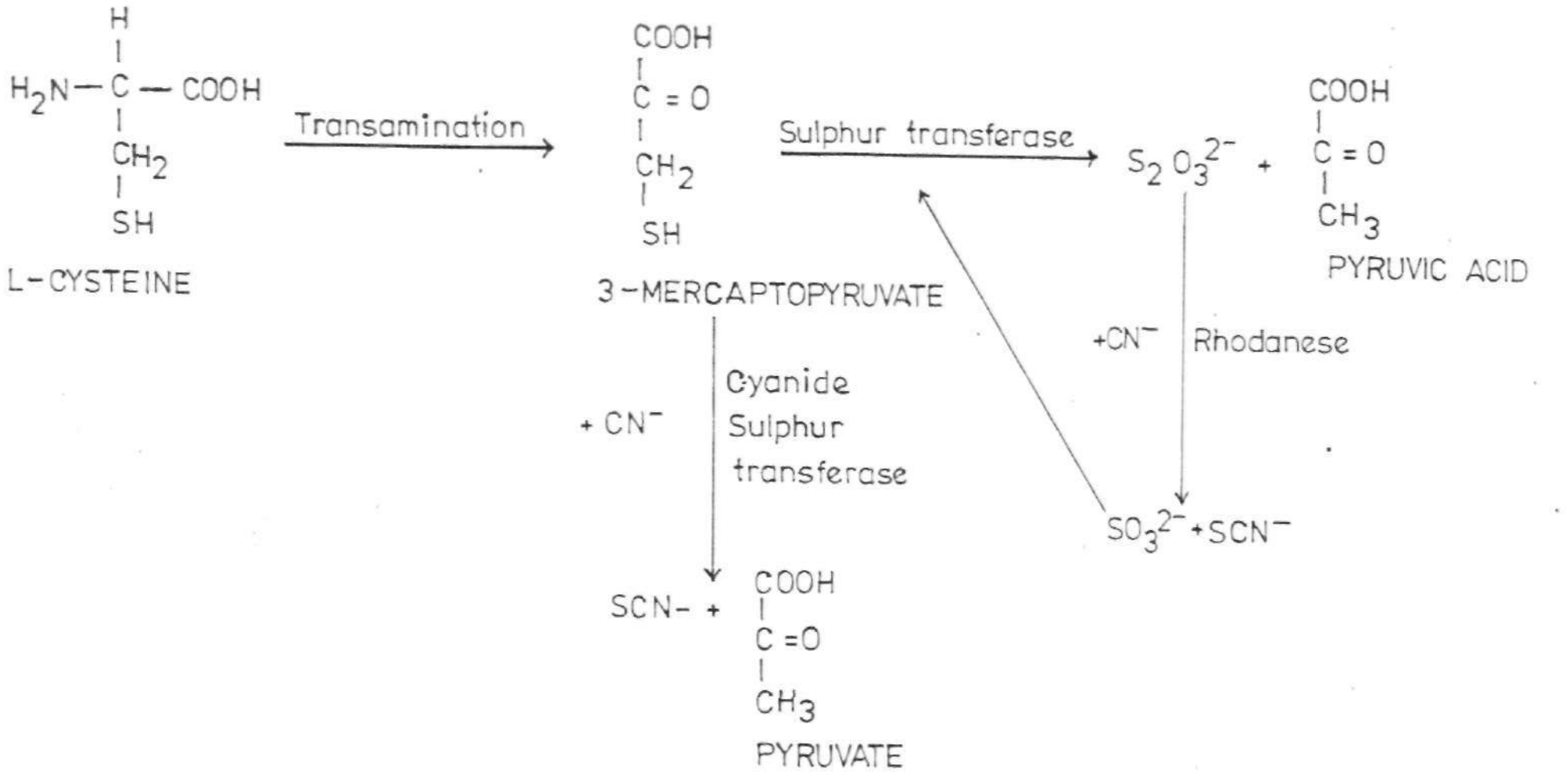


FIG. 5: CYSTEINE AS A SULPHUR SOURCE FOR RHODANESE.

The less important alternative pathway is catalyzed by cyanide sulphur transferase producing thiocyanate and pyruvate from 3-mercapto pyruvate but without the production of S_2O_3 (Figure 5).

The rhodanese pathway represents the chief route of detoxification and occurs in all parts of the body with the largest concentration of the enzymes in the liver. The amount of rhodanese in the liver varies with different animals so that detoxification may be expected to take place at different rates. Himwich and Sounders (1948) found the following for the livers of animals:

Dog, 0.78 - 1.46 mg/g
Monkey, 10.08 - 15.16 mg/g
Rabbit, 7.98 - 18.29 mg/g
Rat, 24 - 28.38 mg/g

This probably explains the results obtained by Mukerji and Smith (1943) that nearly all the cyanide n ingested by rabbits was recovered in the form of thiocyanate in the urine in 24-48 hours, whereas, in dogs, less than 25% was recovered in 7 days. On the other hand, the activity of the enzyme in parts of the brain and the central nervous system seemed to be the same for different species of animals and this may account for the LD 50 for intravenously-injected NaCN to be the same for all species.

Another detoxification process starts with cystine (Figure 7). Cystine reacts with cyanide to form cysteine and B-thiocyanoalanine which tautomerises to 2-aminothidizolidine-4-carboxylic acid or its equivalent 2-imino-4-thiazolidine carboxylic

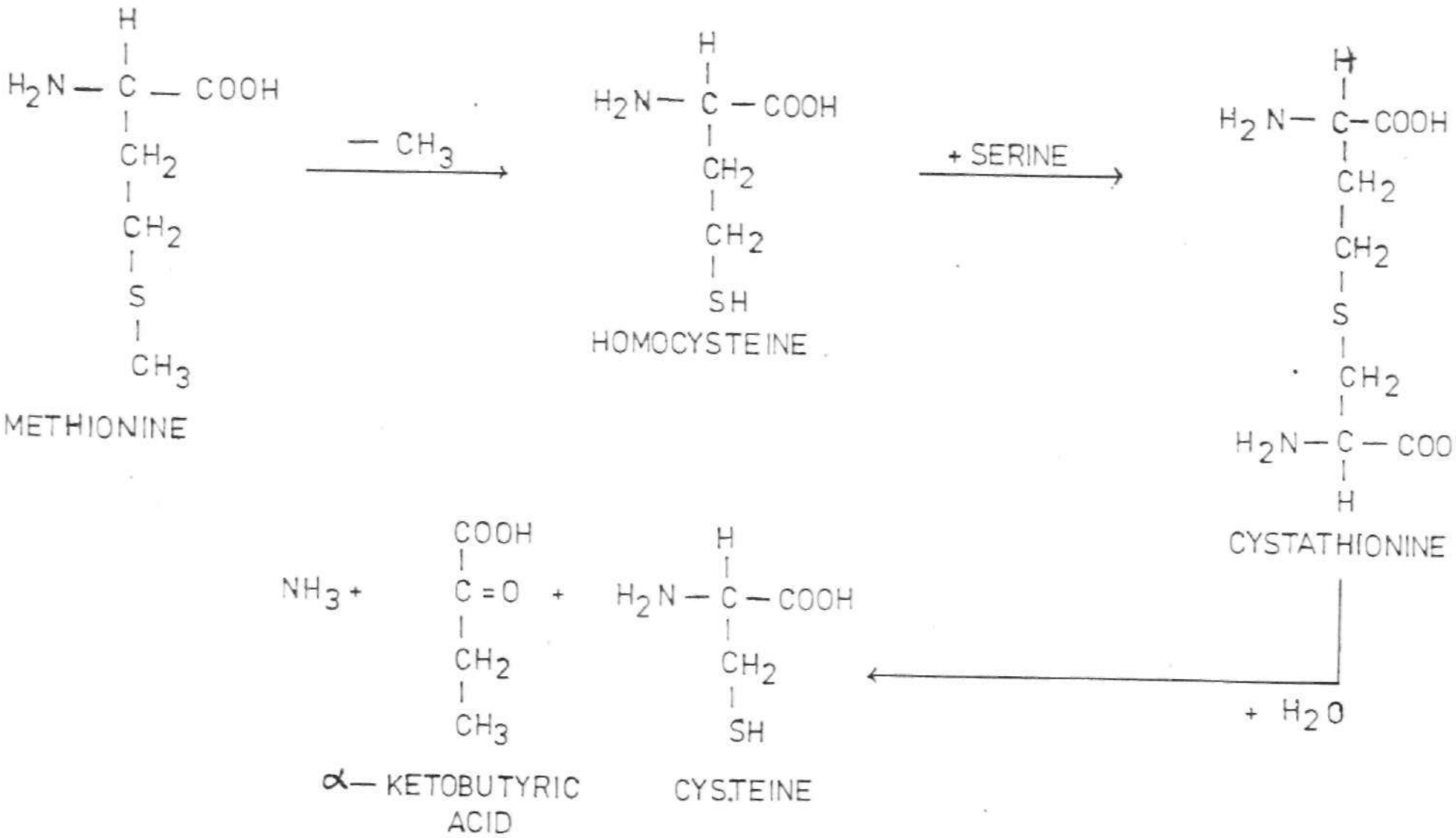


FIG.6: METHIONINE SULPHUR MADE AVAILABLE FOR THIOCYANATE FORMATION THROUGH CONVERSION TO CYSTEINE.

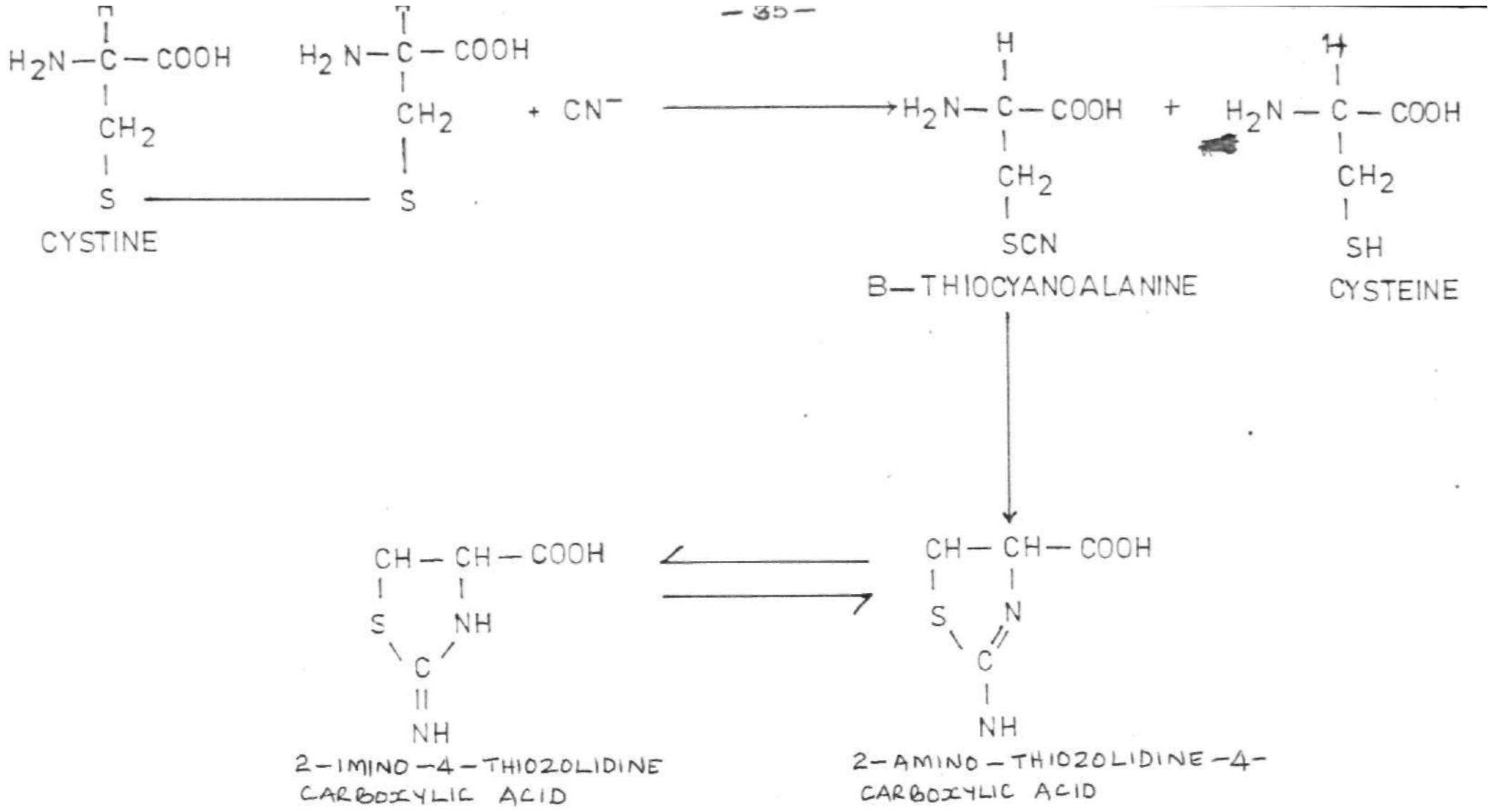


FIG. 7 CONVERSION OF CYANIDE TO 2—IMINO—4—THIOZOLIDINE CARBOXYLIC ACID.

Yet, another indirect detoxification mechanism is that of thyroid gland. The thyroid gland shows some detoxicating effect. In the presence of powdered sheep thyroid the lethal dose of acetonitrite for mice was 1.4 mg/g whereas it was 0.32mg/g for those not fed the powdered sheep thyroid.

PERFORMANCE OF ANIMALS ON CYANIDE-BASED DIETS

Rats

Research conducted by Hill (1977) produced the following results. Young rats fed ad libitum, a diet containing 2,400ppm potassium cyanide showed no obvious signs of distress other than a decrease in weight gain. Similarly, rats fed for 12 weeks a diet deficient in vitamin B_{12} and methionine and containing 1,500ppm potassium cyanide appeared healthy, although weight gains were reduced by the differences of vitamin B_{12} and methionine and by the addition of KCN. Rats receiving fatal doses of linamarin showed characteristic alterations in electrocardiograms similar to those resulting from fatal doses of potassium cyanide. Alterations in several biochemical parameters measured in the blood and in heart tissue were observed in these rats and also in rats receiving lesser but non lethal doses. The severity of the reaction to potassium cyanide and linamarin was strongly influenced by the manner in which they were administered. Mixed with the diet and fed over a period of hours they were tolerated in large amounts; whereas, death resulted from single large doses.

In another experiment with rats on a 50% cassava diet, Olusi et al. (1979) reported the rats had significantly lower birth weight and brain weight and never attained the same adult weight as those of the controls. Adult female rats fed a diet consisting entirely of raw cassava had significantly reduced haematological and biochemical indices. This diet also caused an increased incidence of cannibalism and a significant reduction in the frequency of pregnancy, the average number of the litter and the birth weight among these rats. In addition, there was an increased incidence of neonatal deaths among the offspring which also had poor development, reduced brain weight and an increased tendency towards bitting their litter mates. Those adult female rats fed diets containing 5 and 10g CN/100g laboratory diet surived for more than three months but never became pregnant. They developed enlarged thyroid glands and tumors of the large intestine.

Tewe (1983) also reported that a diet containing 173ppm HCN, increases thiocyanate concentration of urine, serum and amniotic fluid from gestating rats.

Poultry

Experiments with cassava meal in poultry diets date back to 1935 in Philippines (Fuller et al., 1974). Similarly, in Germany and other countries, a significant growth depression was recorded when the level of cassava inclusion was higher than 10-20 percent of the total diet, although in some experiments there was already a tendency to substitute cassava for cereals at levels up to 50 percent. Somewhat better results were obtained

when cassava-based diets were fortified by methionine. Ngoka (1986) reported the inclusion rate of 75% of cassava root meal for broilers without any adverse effect provided fishmeal and methionine are added as supplements. The inclusion of palmoil as per Adeyanju et al. (1979) which promotes methionine utilization may also be helpful.

In his work with chicks, Carew (1981) found that the symptoms of acute toxicity, included respiratory distress, convulsions and collapse with near lethal doses. Chronic toxicity produced the classical symptoms of lethargy, depressed growth rate, elevated blood thiocyanate content, enlarged thyroids. Other less well known symptoms of chronic cyanide intoxication in this experiment were enlargement of the liver and kidneys and elevation of blood thiosulphate concentrations, indicating adaptive improvements in cyanide detoxification.

Swine

The consumption of chopped fresh cassava by growing-finishing pigs varies according to the protein content of the supplement (Gomez, 1979). When a protein supplement was supplied ad libitum with chopped, fresh bitter roots, the pigs consumed an excess of the supplement to compensate for the limited intake of the bitter roots . Experimental results obtained during the different periods of the swine life-cycle suggest that fresh sweet roots are excellent source of energy for swine feeding programmes when properly supplemented with protein, minerals and vitamins.

Rabbits

Much less cyanide research appears to have been conducted with rabbits. In one experiment with New Zealand white and Californian breeds fed corn or cassava-based diets, Fomunyam et al. (1984) found that the rabbits had comparable reproductive, growth and carcass traits, although feed intake and average daily gain were significantly ($P<0.05$) greater for rabbits fed the cassava-based diets. It was more profitable to raise rabbits on the cassava-based diets than on corn-based diets.

The work of Omole and Eshiett (1976) on feeding cassava diets to rabbits shows that the rate of gain decreased with increasing levels of cassava in the ration, although this decrease was not significant untill the ration contained 45% cassava root meal. Preliminary results from the work show that the addition of 0.2% methionine to the control and 45% cassava diets resulted in very slight improvement (0.22 and 0.48% respectively) in liveweight gain over the unsupplemented diets. When the 15 and 30% cassava diets were supplemented, however, there was 5.9 and 3.5% gain over the unsupplemented rations. Addition of methionine to the ration did not improve the efficiency of feed utilization significantly, neither did it affect the quantity of urinary thiocyanate excreted by the animals.

Fomunyan et al. (1983) have also shown that cassava levels up to 45% support good growth in rabbits. Rabbits fed cassava and corn-based diets showed similarity in reproductive growth and carcass traits at the level of cassava inclusion. There was also

similarity in thyroid weights of the rabbits on both diets suggesting that at the levels of thiocyanate were below the threshold causing goitre (Oke, 1973). The similarity for reproductive, growth and carcass traits of the rabbits at 45% level of cassava inclusion suggests that the cyanide did not affect the rabbits or that it was rapidly detoxicated by rhodanese of the liver.

Eshiett et al. (1981) fed New Zealand white does with four diets without cassava root meal or with 15, 20 and 45% cassava root mealy daily, The does were mated and the offspring of the first breeding study were given the same cassava-based diets as their mothers for eight weeks. Number of young per litter at birth and the liveweight of young at the same ages did not differ significantly among treatments. Thiocyanate in urine and blood serum of the mothers was similar among treatments. With the offspring of the same breeding, average rate of gain, feed intake, efficiency of feed conversion and carcass characters were not significantly different among groups.

Ruminants

Generally in feeding trials, it seems reasonable to distinguish between monogastric and polygastric mammals. The stomachs of ruminants are like fermentation tanks, in which breakdown of cyanogenic substances are possible. Ruminants had a higher tolerance to hydrocyanic acid than the other mammals tested (Nestel and Graham, 1977).

Doggett (1970) reported that as little as 0.5g hydrocyanic acid is sufficient to kill a cow. Nevertheless, while clover containing

cyanogenic glucosides equivalent to 129gm of cyanide per 100g dry matter provided no deleterous effects in dairy cows, but plasma thiocyanate increased, producing evidence for metabolic effect (Butler et al., 1957). High levels of cyanogens (180 ppm hydrocyanic acid) and low levels of iodine (±0.05 ppm I) have been reported to cause thyroid dysfunction (Herrington et al., 1971) in sheep grazing cyanodon plectostachys.

The minimum lethal dose of HCN for sheep is 2.4mg/kg body weight (Coop and Blakley, 1949) in agreement with values found for other animals. The minimum lethal dose of lotaustralin; linamarin is 4.5mg HCN/kg body weight; the tolerance to a higher dose can be ascribed to the time required for consumption of the feed to the time required for release of cyanide by linamarase, which may be slower in the rumen because of dilution. Over long periods, sheep were able to detoxify and tolerate 3.9mg HCN/kg per hour for many hours. No evidence of adaptation to cyanide was noticed (Butler et al., 1973). Where the ingestion of forage is relatively slow, as in the normal grazing situation, sheep could well tolerate 15-20 mg HCN/kg body weight per day (Coop and Blakley, 1949).

Shukla et al. (1982) reported that when seven sheep grazed on ratoon crop of jower (sorghum vulgare), they had signs of cyanide poisoning after half hour of grazing. Four of the sheep died within one hour and sodium picrate spot test of rumen contents, histopathological lessions and bright red colour of blood in sheep at the time of death indicated hydrocyanic acid poisoning.

By way of comparison, Corkill (1952) bred a strain of white clover with hydrocyanic acid levels approaching 3,500 ppm, but no evidence of toxicity to grazing sheep was observed, nor were cyanogenic strains less palatable than acyanogenic clover strains. In contrast to white clover, sorghum has often caused death of grazing cattle and is regarded as dangerous to feed when the level in the leaves exceeds 500 ppm (dry weight) (Boyd et al., 1938). However, Rose (1941) recorded that cattle continuously grazing Sudan grass containing up to 1,330 ppm HCN were unaffected, whereas dairy cattle which ate rapidly when put onto Sudan grass were affected by lower levels of cyanide. He also noticed that sheep safely grazed sorghum hybrids which had been poisonous to cattle. Since the maximum level of cyanide in clover pastures might be as high as 1,000ppm, well above a dangerous level in sorghum, it appears that the latter might be more toxic than clover. This could be explained by a faster hydrolysis of the glucoside in sorghum (Butler et al., 1973). Coop and Blakley (1949) noted that the presence of sugars greatly reduced the rate of hydrolysis of lotaustralin, so the quantity of free sugar in the forage may also affect the toxicity by retarding hydrolysis of the cyanoglucoside.

THE ROLE AND NUTRITION OF THE RABBIT

Origin and breeds:

The domestic rabbit is derived from the European rabbit (oryctolagus cunicolos L.) which is member of the order lagomorpha that consist of two families namely, the ochotonidae and the leporidae. The order lagomorpha includes one genus of hare, eight genera of rabbits and one genus of pikas. Oryctolagus cuniculus is the only species of the entire order that has so far been domesticated. This species has been introduced into every country of the world as a source of meat, fur, manure and for use as laboratory animals and pets (Anonymous, 1966). There are more than thirty known breeds of domestic rabbit (Okorie, 1977). The common breeds raised in Nigeria are:

i. Flemish Giant - Steel-grey, light-grey, black, blue and white. Mature weight is about 6kg, and it is used for meat and show

ii. New Zealand - Body is white, red or black, and mature weight is about 5kg.

iii. California - White body, coloured nose, ears, feet and tail. Mature weight is about 4.5kg.

iv. Champagne d'Argent - A dark slate blue under colour, and surface colour is a blue - white or silver with some sprinkling of black hair. Mature weight is about 5kg.

v. English sport - White with black, blue or chocolate spots. Weighs about 4.8kg.

vi. American chincilla - Surface fur grey, under-fur deep blue grey, belly white. Mature weight is about 4.8kg.

Other breeds of the domestic rabbit include Cheekered Giant, Dutch and Belgian.

Rabbit farming is of relatively little importance in developing countries. Ghana is, however considered to have one of the successful rabbit development programmes in this part of the world in form of village small scale production scheme. This had led to the annual consumption of five million rabbits in Ghana (Owen, 1976). Village scale production is also being developed in Zaire, Tanzania and Malawi. In South America, similar efforts are under way in Peru, Brazil and Ecuador, while in the Caribean area and Central America, rabbit production is actively encouraged.

Novelties of the rabbit

The following are considered special attributes of rabbits that have tended to catch the fancy of animal producers (Cheeke, 1980):

i. Rabbits can be fed high-forage, low-grain diets that are largely non-competitive with human food requirements

ii. They can utilize forage protein more efficiently than other livestock. This reflects in the high protein content of rabbit meat as shown in Table 2. In their experiment, Reddy et al. (1977) found that rabbits utilized protein more efficiently than broilers (1.13 and 0.83 kg of body weight gain per kg of protein consumed) (Fekete, 1985).

iii. Unlike any other type of livestock, rabbits can be bred back on the same day they give birth. In the intensive production system, it is theoretically possible to have one litter being raised from weaning to 8 weeks, onelitter with the doe and one litter in the uterus. A litter can be weaned at 4 weeks, and another litter kindled three days later.

Table 2 : The nutrient and energy content per 100g original matter of some types of meat (Fekete, 1985)

Meat	Water (%)	Gross Energy (Kcal)	Protein (%)	Fat (%)
Chicken	74.6	110	21.5	2.5
Rabbit	69.4	158	21.0	2.5
Lamb	66.4	194	19.7	12.7
Lean beef	74.3	116	20.6	3.5
Pork, medium	56.8	295	17.0	25.3
Fish (Carp)	78.4	100	16.0	4.0

iv. Rabbits have a high growth rate (nearly equal to broilers), reaching market weight in 8 weeks.

v. Rabbits exhibit a high feed efficiency. Feed efficiency of 1.04 - 1.46 kg feed/kg gain on practical diets, containing significant amounts of alfalfa meal or other roughage has been achieved.

vi. There is a high degree of genetic diversity in rabbits, both within and between breeds. This variability is useful in breeding programmes, since a wide genetic base of diversity increases the likelihood of success of selection programmes.

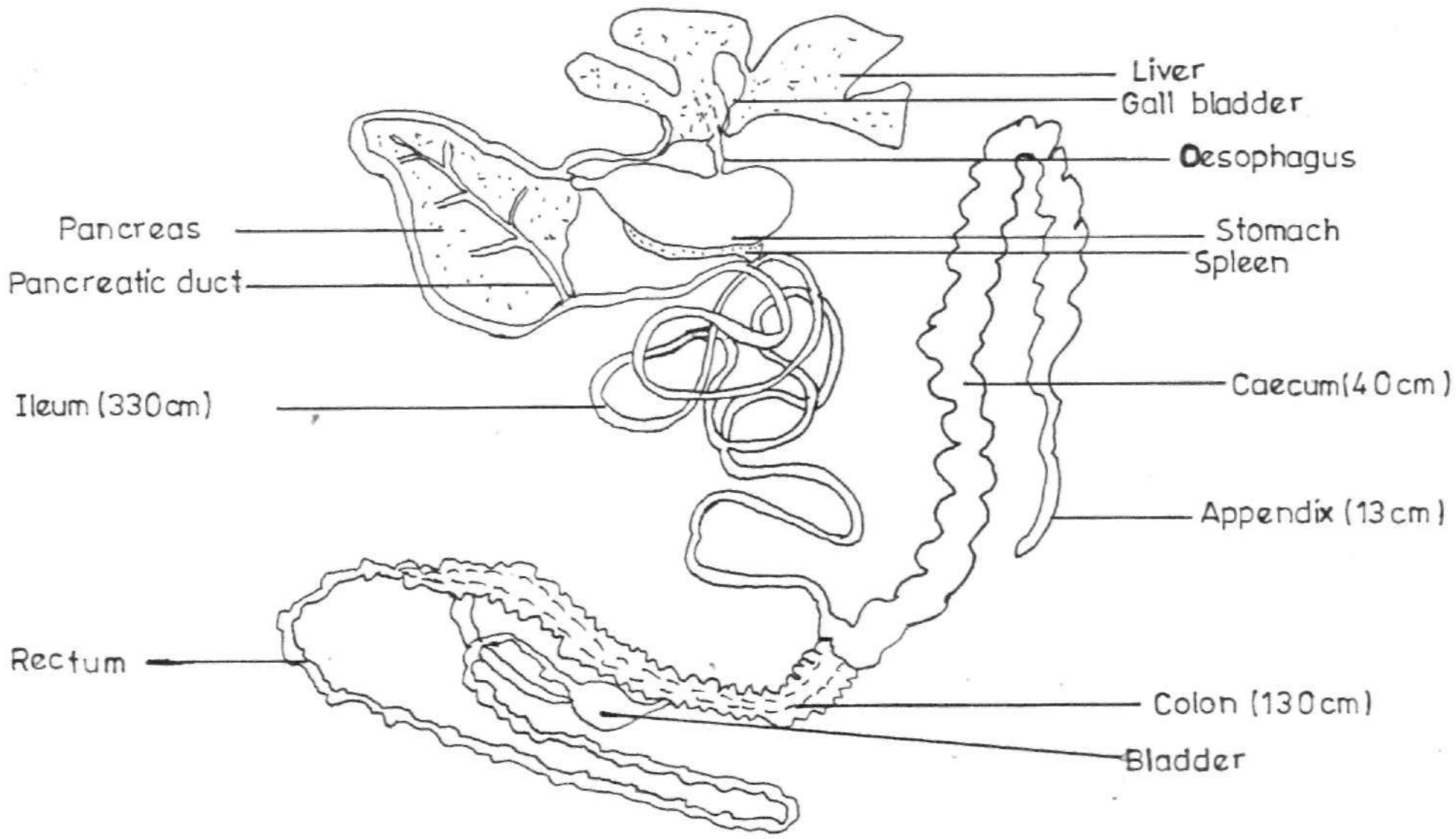
vii. Rabbit meat is a high quality and nutritious product. It has high quality protein (similar to that of chicken) and low fat content. Rao and Coworkers (1979) have stated that because of its extremely low cholesterol and sodium levels (136mg % and 393 ppm, respectively), rabbit meat may play an important role in the prevention of vascular diseases.

viii. From biological point of view, when bred at small scale, the rabbit may be regarded as a "biological refrigerator" since up to its consumption, the meat can be kept alive (Cheeke, 1980). One animal covers the need for one or two meals of a whole family. If the animals are larger, this solves the storage and conservation problems as well.

Anatomy and physiology of the rabbit's digestive tract

The average lengths of some intestinal sections of a mature rabbit (approximately 4 months old, weighing 2.5kg) are as follows:

Figure: 8 Digestive system of a 4-months old rabbit



the small intestine is 330cm; caecum, 40cm; appendix, 13cm ; colon, 130cm (Lebas and Lapl ce, 1972).

The mass of content in the two main reservoirs, i.e. the stomach and the caecum (Fig. 8) of the digestive system of the rabbit is approximately identical, but the relative volume of their different sections differs from that of other domestic animals. It is a striking fact that the cecum and stomach of the rabbit are relatively the largest among the domestic animals, except the forestomach of the ruminants (Pourtsmouth, 1972).

The chemical reaction (particularly the pH) of the different sections of the digestive tract is likewise different. The gastric content is highly acidic, its average pH being as low as 2.2; the content of the small intestine is slightly alkaline (pH 7.2 to 7.9); the content of the caecum has a pH of 6.9 while the proximal third of the colon has a pH of 6.6 viz slightly acidic. The average dry matter of the cecum is 28%. The pH of the small intestine shows very little fluctuation. The low pH and higher acidity of the cecal content compared to that of the small intestine can be attributed to microbial fermentation which converts the fibrous cellulose into volatile fatty acids (Marty and Raynaud, 1966; Vernay and Raynaud, 1975). The total bacterial count in the caecum is $10^9/g$ on the average; $10^8/g$ in the colon and rectum; and still lower in the jejunum.

Nutrient requirements of the rabbit

Table 3 is a summary of the nutrient requirements of classes of rabbits (Lebas, 1983). Diets high in readily-available carbohydrates when fed to rabbits, especially fryers, result in high losses caused by enterotoxemia or enteritis complex (mucoid enteritis), which is related to carbohydrate overload of the hindgut (Cheeke and Paton, 1980). It has been suggested that enteritis can be prevented by feeding indigestible fibre. It is argued that fibre has a scrapping effect on the lining of the intestine, and that in the absence of this stimulating effect of fibre, the intestinal cells lose their vitality leading to bacterial invasion and enteritis. But indigestible dietary fibre is important in rabbit nutrition not only for the prevention of enteritis, but also for maximum growth and to prevent fur-chewing. An additional effect of fibre in rabbit nutrition is that it may be involved in cecal impaction. Pote et al. (1980) found that the growth rate of fryers was lower on a high energy corn-soybean meal diet than when a fibre source (alfalfa meal) was added. Hence, optimum crude fibre level of about 14 to 15% should be maintained in practical diets. Cheeke and Paton (1981) suggested a possible involvement of high (over 22%) dietary fibre levels.

Rabbits are well-adapted to the use of low energy diets. Specific energy requirements have not been well studied but a dietary level of 2,500 Kcal D/kg of feed provides for adequate growth and reproductive performance (Cheeke, 1984). Energy requirements are, however, greater for lactation than for other

Table 3 : Nutrient requirements of rabbits of various status (Lebas, 1983)

Constituent	Unit	4-12 week old (young)	Nursing doe + young	Pregnant doe (non-nursing)	Adults maintained including males
Digestible energy	Kcal/kg	2,500	2,800-2,900	2,500	2,100-2,200
Crude protein	%	15-17	18-19	15	13
Methionine + Cystine	g/kg	5	6	-	-
Lysine	g/kg	6	7.5	-	-
Crude fibre	%	13-15	12	14	15-16
Fat (min)	%	3	14	3	2
Calcium	g/kg	5	11	8	6
Phosphorus	g/kg	3	8	5	4
Nacl	g/kg	10	10	10	-
Vitamin A or vitamin D	IU/kg	6,000	12,000	10,000	-
Vitamin E	IU/kg	900	900	900	-
Vitamin K	mg/kg	50	50	50	50
	mg/kg	-	3	2	-

productive functions in rabbits (Lang, 1981b) because milk production relative to body weight is high and rabbit milk has a high energy content (8.4 - 12.6 MJ/kg). Thus, it may be desirable to employ high energy lactation diets.

The protein and amino acid requirements of rabbits are higher for lactation than for growth. Protein requirements are 15-16% for growth and 18-20% for lactation (Omole, 1982). Cheeke et al. (1985) carried out an experiment on protein requirements for optimal growth and reproduction and conclused that a level of 17.5% crude protein seems adequate for satisfactory reproductive performance and growth of commercial meat rabbits. Rabbits also require dietary essential amino acids. Microbial synthesis of protein in the cecum makes a minor contribution to the amino acid needs of growing rabbits, but this phenomenon is more important in adults (Lang, 1981a).

The vitamin needs of rabbits are generally quite easily met because of the nature of common rabbit diets and of the digestive tract. Conventional rabbit diets usually contain 30-60% of dried forage such as alfalfa meal. If this is of good quality, the requirements of fat-soluble vitamins will be met (Cheeke, 1984). The B vitamins are provided by both the diet and from consumption of cecotropes (Lang, 1981a). Infact the major function of coprophagy in the rabbit appears to be the provision of B vitamins synthesized by bacteria in the cecum.

The provision of 0.5% trace mineralized salt in the diet will provide adequate quantities of these nutrients. Alfalfa meal, a major constituent of most commercial rabbit feeds, is an excellent source of calcium and potassium (Cheeke, 1984). Grain by products such as wheat bran are excellent sources of phosphorus. Hence, a combination of alfalfa meal and grain by products as major ingredient can satisfy the calcium and phosphorus requirement.

Feed utilization by rabbits

The digestion coefficient of rabbit feedstuffs and nutrients vary tremendously. The rabbit utilizes fats very well (90-100%), proteins, fairly well (60-80%), fibre rather poorly (20-40%). Accordingly, its crude fibre digestion is inferior to that of the ruminants and horses, but better than that of poultry and swine (Slade and Hintz, 1969). The digestion coefficient varies with factors related to the animal itself (individual, age, sex, variety, biological state) and to the feed (ration composition, feeding level, additives).

Cecotrophy or biological coprophagia

The basis of the cecotrophy (pseudo-rumination) of the rabbit is that they produce two different kinds of faeces in their intestinal canal, one of which the animal consumes regularly. The faeces suitable for consumption is called soft faeces, or since it originates from the caecum, cecotroph; while the other which the rabbit does not eat is called hard or benuine faeces. Both types of faeces differ in their chemical composition (Table 4).

Table 4 : The composition of the two types of rabbit faeces (Fekete and Bokori, 1984)

Constituents	Hard faeces	Soft faeces
Dry matter (%)	52.7	38.6
Crude protein (%)	15.4	25.7
Ether extract (%)	3.0	5.3
Crude fibre (%)	30.0	17.8
Ash (%)	13.7	15.2
Nitrogen free extract (%)	37.9	36.0
Gross energy (Mkal/kg)	4.34	4.53

Cecotrophy cannot be observed in new-born rabbits. It develops gradually from the third week of their lives onwards, parallel with the transition to solid feed. Cecotrophy contributes to the vitamins B and K supply, provides essential amino acids, continuously regenerates the gastric microflora and ensures a more efficient and more economical utilization of nutrients, particularly of proteins, and explains why rabbits are to, a large extent, practically insensitive to the quality of feeding proteins. The importance of coprophagy is shown by the fact that if it is prevented, the growth rate of the rabbits drops by 12 to 25% (Barnes et al., 1963) and they show symptoms even of vitamin B defficiency (Kulwich et al., 1953).

Problems and Prospects of rabbit production

The rabbit industry is beset with many problems that have tended to militate against its development. The problems have continued to relegate rabbit farming to what could be termed a "backyard enterprise". The most serious problems include - diseases, high feed cost, high labour requirements and market stability.

The incidences of the two most important diseases, enteritis or snuffles and respiratory diseases like pneumonia have, however, been greatly minimized. Already the Oregon State University Rabbit Research Centre has developed a rabbit strain that is resistant to pasteurella multocida, the causal bacteria of snuffles.

With respect to high cost of feeding, it is considered that since rabbit feed costs are basically similar to those of other livestock, the relative differences in profitability of poultry and rabbit productions, for example are due to factors other than costs.

There are two alternatives to the high labour requirements - reduce labour costs through automation, or raise rabbits where labour is cheap, such as in developing countries. These may explain why Chinese - imported rabbit into North America are cheaper than their locally - produced rabbit.

Market stability or otherwise is a universal phenomenon that affects all industries. In short, it is a normal feature of a dynamic economic system.

Thus, inspite of the enumerated problems, there is some optimism for the future, granted the present concerted efforts by researchers. Afterall, many decades back, the poultry industry was hardly any better (McGinty, 1985).

PERFORMANCE OF GROWING RABBITS ON MALTED SORGHUM

SECTION I

1. ABSTRACT

Two experiments were conducted to assess the performance of growing rabbits on malted sorghum (sorghum biclor L.) which contains the cyanogenic glucoside, dhurrin, Sorghum was malted using the technic of Wu and Wall (1980). The first experiment conducted with weanling (4 week old) rabbits, had two treatments with six replications per treatment. The diets were isonitrogeneous and isocaloric. Sorghum inclusion rate was 26%. Feeding lasted for six weeks and records of performance were taken. In the second experiment, rabbits were about 9 to 10 weeks old. There were three treatments with four replications per treatment. Sorghum inclusion rate was 32% in all the diets. Feeding lasted for five weeks and growth parameters were recorded.

Proximate and cyanide analysis showed that while malting improved the nutritional status of sorghum, particularly protein, it along side raised total and free cyanide contents from 22 to 963 and 22 to 168 ppm respectively. But even at this level, the rabbits did not show signs of cyanide toxicity. Rather, the rabbits on malted sorghum-based diets had better weight gain and feed efficiency. Malting did not affect feed intake and carcass quality. Generally rabbits performed better in the first experiment than in the second. This and other reasons sugrested that 26% rate of sorghum inclusion was preferable. Removal of roots and shoots from malted sorghum depressed performance.

2. INTRODUCTION

Limited land resources and the ever-increasing human population have tended to dictate that emphasis be shifted to indoor and confined management of animals. With its ability to survive almost exclusively on household food wastes, the rabbit is apparently favoured with the new trend.

The rabbit (Oryctolagus cuniculus) has great potential as a meat-producing animal and can produce more meat from forage-based diets than any species of livestock (Cheeke and Patton, 1980). Indeed the rabbit is one of the cheapest producers of meat but hitherto neglected in Nigeria. According to Deblas and Garvey (1975), the rabbit is suitable to raise for meat because of its high fecundity and prolificacy, short generation interval and high feed conversion efficiency. Rabbit meat is nutritionally comparable to that of poultry, and at this time in Nigeria when the high cost of commercial poultry feeds has sent many producers out of the industry, the rabbit is a potent alternative considering its ease of management.

Sorghum (sorghum bicolor L. Moench) ranks fourth among the world's leading cereals. In Nigeria, it ranks first and occupies about six million hectares of land. Its agronomic advantages include ease of propagation and lesser fertilizer requirement compared to corn. Sorghum contains more protein and carbohydrates, less crude fibre and fat than corn (Hulan and Proudfoot, 1983; Okoh et al., 1982). It is also higher than corn in apparent metabolizable energy and all amino acids considered essential

for chickens (Hulán and Froudfoot, 1983). Ross and Webster (1960) found that feeding grain sorghum to most livestock species is comparable to corn. Sorghum contains tannin which binds lysine and hence makes lysine a deficient amino acid in sorghum-based diets (Schang, et al. 1986). Tannin is also found to reduce the metabolizable energy of the diets and increase blood cholesterol (Vohra et al., 1986). Malting (Wu and Wall, 1980) and reconstitution (Mitaeur et al., 1985) are known to reduce tannin content (Kubiczek et al., 1984).

As malting proceeds with reduction in tannin and an increase in amino acids, there is however, a corresponding increase of a cyanogenic glucoside, dhurrin in the grain. This cyanogenic glucoside, according to Doggett (1970) is a toxic substance which yields hydrocyanic acid (HCN) on hydrolysis in the body of the animal (Okoh et al., 1987). Apparently the consumption of sorghum sprouts or products made from them may be hazardous because of its high cyanide content. Hydrocyanic acid also(called prussic acid) is a highly-toxic substance, 200ppm being a lethal dose for adults, resulting in death within a few minutes. In small doses, the cyanide is detoxified to thiocyanate by the enzyme, rhodanese which makes use of sulphur amino acids as the sulphur donors, thereby making methionine the second limiting amino acid in sorghum-based diets.

The purpose of this study is to:

(1) assess the response of the rabbits in terms of feed consumption, weight gain and feed efficiency in order to determine if the cyanide

formed during malting interfers with the efficient utilization of the improved nutritional attributes in the malted grain;

(2) see if the removal of roots and shoots from malted sorghum grains will affect the performance of rabbits feeding on it.

3. MATERIALS AND METHOD

Malting of sorghum

Two procedures were tested for their suitability for sorghum malting. These were the Wu and Wall (1980) and the Mitaru et al. (1983) methods.

Wu and Wall (1980) method

About 3kg of sorghum (mori variety) was soaked at a time with about 3 volumes of distilled water overnight, with two changes of water during the day to remove dirt and extra husk. The wet grain was then soaked in 0.2% formaldehyde solution for 40 minutes to retard mould growth during germination. The soaked grain was then washed four times with distilled water and soaked in water for 20 minutes to remove residual formaldehyde. The wet sorghum was then spread out thinly on filter papers saturated with distilled water in a container with plenty of air space and covered with cellophane. The grain was then allowed to germinate at room temperature for 4 days. As germination progressed, grains were checked for the appearance of mould. A few mouldy grains were detected on the fourth day and these were normally discarded.

This method was used in the large-scale malting of sorghum for the experimental diets. In this case, however, the grains were spread thinly on cellophane sheets, previously spread on tables in a room and covered with some of the sheets while allowing for a film of air above the grains. The grains were turned gently and distilled water was sprinkled on them for three days to prevent dehydration and provide aeration for the grains. The malting process continued for four days. The sprouted grains were then spread on a concreted surface to dry by sunrays. The

dried grains were then ground and mixed into the rabbit diets.

To remove shoots and roots in the second experiment, sprouts were rubbed with the palm of the hand against the sieve. Most of the roots and shoots readily passed through the pores of the sieve. The small amount (of roots and shoots) not removed by the above process were readily cleared by winnowing since they were very light.

Mitaru et al. (1983) Acetate-propionate treatment:

Preliminary handling before chemical treatment is as per Wu and Wall (1980). Moisture content of grain was then raised by adding 25% (w/w) distilled water. An acetic and propionic acid mixture (60/40, v/v) was also added at 2% w/w. The ingredients were properly mixed until the sorghum grain imbibed all the water which was indicated by lack of lump formation by the grain. The treated grains were stored as in previous method for 1-4 days' germination.

NB: The 2%, w/w mixture of acetate-propionate was later reduced to 1%, 0.5% and even 0.1% without germination and without mold formation. This method was therefore confirmed unsuitable for our purpose.

Animal Experiment :

This study consisted of two treatments with six rabbits per treatment. Treatment I had three New Zealand White and treatment II three California White rabbits, with an average weight of 346.7g. The rabbits were randomly allocated singly to individual cages.

The diet used in this first study were formulated to meet 20% crude protein specification. The two diets contained the same amounts of wheat offals, groundnut cake, molasses, blood meal, salt and vitamin-mineral premix. The only differences between the two diets is that one contained malted sorghum while the other contained unmalted sorghum. The components of the two diets are shown in tables 5 and 6.

The rabbits were fed ad libitum in plastic feeders (which were later changed to metal feeders) and they had access to water in plastic waterers. Both the feeders and waterers were tied to the cages to prevent wastage. To minimise feed wastage, wire mesh was securely fixed on top of each feeder leaving a small space through which the rabbit's head could enter.

Table 5 : Percent ingredient composition of the malted and unmalted sorghum-based diets

Ingredient	Treatments Diet I (malted)	Diet II (unmalted)
Wheat offal	47	47
Groundnut cake	20	20
Unmalted sorghum	-	26.25
Malted sorghum	26.25	-
Salt	0.5	0.5
Bone meal	3	3
Molasses	3	3
Vitamin/Mineral Premix*	0.25	0.25

Calculated Analysis of Nutrients

Nutrient	Diet I	Diet II
Crude protein %	19.08	18.92
DE (Kcal/kg)	2718	2701
Crude fibre %	8.98	8.74
Ether extract %	3.37	3.20
Calcium %	0.470	0.434
Phosphorus %	0.781	0.821
Lysine %	0.92	1.20
Methionine + Cystine %	0.616	0.604

*Contents per kilogramme: Vit A stabilized - 6670,000I.U., Vit. D (stabilized) - 1,500,000I.U., Vit E (stabilized) - 3,340I.U.; Vit. K (stabilized) - 1,340mg; Vit B_2, 3000mg; Vit B_6 - 2000mg; Niacin - 14,670mg; Calcium D-Pantothenate - 4000mg; Vit B_{12} - 8mg; Choline Chloride - 133,400mg; D 0.7 - 6,600mg; Manganese - 53,340mg; Iron - 33,340mg; Zinc - 26,670mg; Copper - 1,600mg; Iodine - 934mg; Cobalt - 134mg; and Selenium - 34mg.

Apart from the diets, all other factors were similar throughout the experimental period. The experiment lasted 42 days. The feed for each rabbit was weighed out each day and put into the feeders. Wasted feed were recovered and weighed. The rabbits were weighed weekly. From the data collected, weekly and cummulative weight gain, feed intake and efficiency of feed conversion (kg feed/kg bgain) were computed. At the end of the experiment, three rabbits from each treatment were taken to the meat laboratory and slaughtered for carcass analysis. The measurements taken were liveweight, empty body weight and weight of the various internal organs. All data collected were analyzed statistically by the one-way analysis of variance (Steel and Torrie, 1960).

Proximate analysis of grain sorghum

Proximate analysis of both malted and unmalted sorghum was done. Consequently, percent composition of crude protein, dry matter, moisture content, ash and ether extract were determined. This was to assist to determining the specific nutrient contribution of sorghum in each diet.

4. RESULTS

[illegible]

Malting of sorghum

At the end of the fourth day of malting, there was good germination rate, averaging about 95%. Very few mouldy grains observed were removed before and on the fourth day just before drying.

Proximate composition and cyanide content of malted and unmalted grain

The proximate composition of both malted and unmalted sorghum are presented in Table 7. Of particular interest is the increase in crude protein level from 9.38% to 10.27% on dry matter basis during the four-day germination. Ether extract was found to have decreased during germination quite remarkably. Total and free cyanide increased during the four-day malting from 22 to 795 ppm and 22 to 169 ppm respectively.

Feed efficiency, Daily weight gain and Daily feed intake

The statistical analysis showed there was significant difference ($P<0.05$) in the feed efficiency of the two diets, malted sorghum-based diet having a lower value as shown in Table 8. The decrease in feed efficiency was about 28.4%, dropping from 4.30 to 3.35 in the unmalted grain-based diet.

Although the mean of the daily feed intake of Diet I (51.65g) is slightly lower than that of Diet II (53.71g), the difference between the two was not statistically significant ($P<0.05$).

Table 8: Daily feed intake, weight gain, feed efficiency, total and free cyanide intake by rabbits fed on malted and unmalted sorghum-based diets

Treatments	Total CN consumed (g) per rabbit in 6 weeks	Free CN^- consumed (g) per rabbit in 6 weeks	Daily feed intake[b] (g/day)	Feed efficiency (feed/gain)[a]	Daily weight gain (g/day)[a]
Malted sorghum-based diet	0.39	0.08	51.65	3.35	15.16
Unmalted sorghum-based diet	0.02	0.02	53.71	4.30	13.41
S.E.D.			2.960	0.332	1.071

a = Statistically significant
b = Not significant ($P>0.05$)

The difference in the mean daily weight gain of rabbits on the two diets were statistically significant ($P < 0.05$) with Diet I having higher value (15.61g/day) than Diet II (13.41g/day).

Carcass Analysis

The result of analysis is shown in table 9. It shows that there was no significant difference ($P < 0.05$) between carcasses of rabbits fed the two separate diets.

Cyanide consumption

Values for cyanide consumption by the rabbits are shown in table 7. Average total cyanide consumed was 0.39g/rabbit in six weeks from Diet I and 0.02g/rabbit in six weeks from Diet II. This means that average daily total cyanide consumed per rabbits in six weeks on Diets I and II were 0.009g and 0.0005g respectively. The average on diets I (0.08g)and II (0.02)g) were also significant ($P < 0.05$). This gave average daily free cyanide consumed to be 0.002g for Diet I and 0.0005g for Diet II.

Table 9: Carcass analysis of rabbits fed malted and unmalted sorghum-based diets.

Variable	Treatments[b] Malted	Unmalted	SED
Liveweight (g)	925.7	1083.3	149.82
Empty body weight (g)	707.3	786.3	121.33
Empty body weight as percentage of liveweight	71.5	72.5	1.38
Roasted weight (g)	603.3	665.7	110.91
Roasted dressing %	60.9	61.0	2.20
Stomach weight (g)	69.0	72.0	10.50
Cecum weight (g)	72.3	79.7	9.18
Large intestine weight (g)	29.0	23.0	8.08
Small intestine weight (g)	42.0	44.7	3.07

b = Not significant ($P>0.05$).

5. DISCUSSION

Malting of sorghum

From the results, it is evident that the acetate-propionate chemical treatment is not an appropriate malting technique since the grains did not germinate even after further dilution of the acid. It nevertheless proved to be an effective anti-fungal agent since there was no mould growth at any stage. This medium may be tested on other experiments other than malting.

The formaldehyde treatment as recommended by Wu and Wall (1980) was consequently confirmed as an appropriate malting technique as adequate malting was observed.

Proximate composition and cyanide content of the malted sorghum grain

The higher DE (digestible energy) level in the malted sorghum grain as shown in table 8 (3,454.70 Kcal/kg) is explained on account that some fat was converted to energy in order to augment energy that is burnt in the process of germination. It is the unutilized surplus energy from this reaction that tends to slightly increase the energy level in the malted grain as compared to that of the unmalted grain (3421.30 Kcal/kg). This reflects in lower fat value in the malted grain. This is somehow contrary to the findings of Wu and Wall (1980) who reported that fat and ash dontent of sorghum grain did not show any trend as germination proceeded. The roots and shoots normally have high energy concentration which is

used to assist the high energy requirement of the meristematic regions of the grain. Their removal (as in the case of malted grains with shoots and roots removed) naturally made the grains have relatively lower DE level (3406.26 Kcal/kg).

Protein levels were also found to be higher (10.27%) in the whole malt than in the unmalted grain (9.38%). This is in agreement with previous research findings (Dalby and Tsai, 1976; Wu and Wall 1980 and Kubiczek et al., 1984). When roots and shoots were removed, the protein value dropped drastically to 6.25% indicating that protein is concentrated in the roots and shoots during germination. These findings therefore, confirm the fact that malting is desirable and beneficial to the animal.

The change in crude fibre contents were not so pronounced. The relatively higher level in the ones where roots and shoots were removed is because the roots and shoots which comprise the easily digestible portions are no more (thereby increasing slightly the fibre : easily digestible carbohydrate ratio).

The values for total and bound cyanide which were 963 ppm and 795 ppm respectively are close to the findings of Parasiuk and Bills (1984) which was 613 ppm hydrocyanic acid. Also, the increase in total and free cyanide during malting from 22 to 963 and 22 to 168 respectively is in line with the findings of Conn (1969) who reported that on germination, cyanide content of sorghum increased. Since dhurrin is the cyanogenic glucoside in sorghum (Gyemuga, 1968), it implies that the latter contributed most (about 83%)

of the total cyanide in sorghum. The toxicity of malted sorghum therefore, largely depends on the rate of metabolic hydrolysis of dhurrin (Okoh, 1978). Ordinarily, if fed alone, 613 ppm hydrocyanic acid is above lethal dosage. Besides the hydrolysis factor, this danger was further lessened during feeding as the rate of sorghum inclusion was pegged at 32% of the three diets in the experiment. When shoots and roots were removed, the bound cyanide levels in the grain was about the same with the unmalted grain. This shows that the cyanide is usually concentrated in the meristematic regions.

Average daily feed consumption, weight gain and feed efficiency

Although mean daily feed intake in both experiment I and II showed there was lower feed consumption in the whole malted sorghum-based diets, the difference between treatments were not significant ($P>0.05$). This means malting has very little influence on feed consumption by rabbits at both early and later stages of growth.

In the first experiment, mean daily weight gains in diets I and II (Table 8) were significantly different. In both experiments therefore, the treatments with malted sorghum produced better rate of gain. These results can be associated with the

higher protein and amino acid levels found in malted sorghum as reported by Wu and Wall (1980) and Dalby and Tsai (1976).

The feed efficiency values obtained in both experiments I and II largely reflected the pattern of results for daily weight gain in the two experiments. As expected, the difference in feed efficiency of the malted sorghum based diets compared with other treatments were better, significantly ($P<0.05$) in the first experiment. The feed efficiencies of 8.85, 5.15 and 7.14 obtained for treatments I, II and III respectively in the second experiment is definitely very poor when compared to values of 4.30 and 3.35 for treatments II and I respectively in the first experiment. These levels therefore produced diets with much higher crude fibre levels and much lower energy levels (which are desirable) in the first experiment as compared to diets with much lower crude fibre and of course higher energy levels in the second experiment. The composition of diets in the second experiment should naturally depress performance in accordance with the findings of Cheeke and Paton (1980). A diet with so high a level of easily digestible carbohydrates has besides, depressing growth, also caused high losses in fryers as a result of enterotoxemia or enteritis (Cheeke and Paton, 1980). Crude fibre level of 14 to 15% or even 22% have been recommended (Cheeke and Paton, 1981a) as against about 7% in diets of the first experiment.

The feed efficiency of 3.35 obtained in Diet I of the first experiment is slightly better than 3.5 obtained by Harris *et al.* (1983) with Kudzu diet on rabbits. Better results have been obtained when peanut and alfalfa (2.6 and 3.2 respectively) were fed by the same author. The rabbit can attain feed efficiency of 1.04 to 1.46, comparable to that of poultry, under ideal conditions (Cheeke, 1980). An ideal rabbit diet, according to Laing (1981a) should contain dry forage of between 30-60% which requirement our diet did not meet. This was because in order to balance other nutrients following the inclusion of 32% sorghum, the energy level was unavoidably raised.

The improved performance of rabbits on the malted sorghum-based diets confirmed that the nutritive quality of sorghum improves upon malting (Berk, 1976; Yakovleva, 1983). In esssence, the benefits of malting sorghum which include increased availability of lysine, methionine, tryptophan and other amino acids (Wang and Fields, 1978) and that of reduced tannin content as reported by Kubiczek *et al.* (1984) were fully utilized by the rabbits. This is particularly important since tannin in sorghum has been reported (Ford and Hewitt, 1979) to lower protein digestibility in pigs.

The feed efficiency of 7.14 in treatment III when compared with 5.15 and 8.85 in treatments II and I respectively (of the second experiment) showed that the beneficial effects of malting were only partially retained when roots and shoots were removed. This showed that removal of roots and shoots from malted sorghum lowered its nutritive value.

Cyanide consumption

Calculated cyanide consumption by the rabbits in the first experiment showed that there was increment in cyanide consumption with increased intake of Diet I.

Carcass analysis

The results showing that there was no significant difference ($P/\epsilon.05$) between the carcass analytical values for the two treatments in Experiment I is similar to the findings of Eshiet et al. (1981). Although Tewe (1984) reported a contrary finding with cassava-based diets on rats, the modes of cyanide intake in each case may vary. Thus it appears feeding malted sorghum-based diets did not have any meaningful effect on carcass character of weanling rabbits.

6. CONCLUSION

The sorghum malting technique of Wu and Wall (1980) which was adopted in the experiments here proved an effective method for germination of sorghum even on commercial basis. Its ability to keep low the rate of mould formation even on commercial basis recommends it for use by breweries and other industrial concerns.

Although cyanide content of the malted sorghum were found to be quite high (963 ppm total cyanide), its deleterious effect were apparently avoided by ensuring that the maximum level of sorghum inclusion (32%) was below the lowest toxicity threshold of about 45% inclusion rate for cassava-based diets (Eshiet et al., 1981). And since sorghum has a much lower cyanide value (about 963 ppm) than the in cassava (about 2,400 ppm), the dangers were much less. Again, bound cyanide constituted about 83% of the total cyanide in the sorghum, and since toxicity of cyanogenic glucosides largely depends on the rate of metabolic hydrolysis in the animal (Okoh, 1978), the real dangers of toxicity in our experiments were further reduced.

The fact that malting did not affect feed intake in both experiments is a welcome finding. Thus, even at the inclusion rate of 32%, malted sorghum did not affect rate of feed intake. Malting however, improved both mean daily weight gain and feed efficiency of rabbits. The best results of 15.61g/day and 3.35 for mean weight gain and feed efficiency respectively compare favourably with results of 15.80g/day and 3.3● respectively elsewhere (Harris et al., 1983).

The experiments have confirmed rabbits utilized the nutritional benefits of malted sorghum. In particular, the benefits of increased protein and amino acid utilization as well as decreased tannin content were all combined.

The roots and sheets were found to be the 'custodians' of the benefits of malting sorghum. Hence their removal lowered its nutritional quality close to that of the unmalted grain, as overall performance of the rabbits suggested. Although cyanide is concentrated in the roots and sheets (Ikediobi et al., 1987), they are better left intact since their removal drastically reduced crude protein level from 10.27% to 5.25% and decreased daily weight gain.

SECTION II

THE IMPLICATIONS OF FEEDING CYANOGENIC MALTED SORGHUM TO RABBITS.

1. ABSTRACT

An experiment was conducted to study metabolic fate in the rabbit, of dhurrin, present in malted sorghum (Sorghum bicolor L.). The malting of sorghum was done using Wu and Wall (1980) technique. The experiment was done with rabbits averaging 9 to 10 weeks old. There were three treatments with four replications per treatment. The diets were isonitrogenous and isocaloric. Sorghum inclusion rate was 32% across the diets. Feeding lasted for five weeks. Urine was collected in the 3rd and 5th weeks and faeces in the 5th week. These were analysed for cyanide and thiocyanate (SCN^-) contents. On the last day of feeding, three rabbits per treatment were slaughtered and plasma, stomach, small and large intestines and caeca were taken and stored for analysis of cyanide and thiocyanate.

Proximate analysis showed that malting improved the nutritive status of sorghum, particularly protein. The levels of total and free cyanide increased from 22 to 963 ppm and 22 to 168 ppm respectively. But because bound cyanide which constituted about 83% of total cyanide had to be first hydrolyzed to exert its toxicity, the dangers of the high cyanide levels were already lessened. Analysis of physiological indices confirmed previous findings that urine is the major route of cyanide carbon elimination in the form of thiocyanate. Chronic cyanide intake had cummulative effect on body thiocyanate concentration. The inclusion rate of 32% malted sorghum in the diets was harmless to the rabbits.

2. INTRODUCTION

Sorghum (Sorghum bicolor L. Moench) ranks fourth among the world's leading cereals. In Nigeria, it ranks first and occupies about six million hectares of land. Sorghum contains more protein and carbohydrates, less crude fibre and fat than corn (Hulan and Proudfoot, 1983; Okoh et al., 1982). It is also higher than corn in apparent metabolizable energy and all amino acids considered essential for chicken's (Hulan and Proudfoot, 1983). Feeding tests on most livestock species has found it to be comparable to corn (Ross and Webster, 1960). Sorghum contains tannin which binds lysine and hence makes lysine a deficient amino acid in sorghum-based diets (Schang et al., 1986). Tannin is also found to reduce the metabolizable energy of the diets and increase blood cholesterol levels (Vohra et al., 1986). Malting (Wu and Wall, 1980) and reconstitution (Mitaru et al., 1985) are known to reduce tannin content of sorghum. In addition, malting also increases lysine contents of the grain (Kubiczek et al. 1984).

As malting proceeds with reduction in tannin and an increase in amino acids, there is, however, a corresponding increase of a cyanogenic glucoside, dhurrin in the sorghum grain. This cyanogenic glucoside, according to Doggett (1970) is a toxic substance which yields hydrocyanic acid on hydrolysis in the body of the animal (Okoh, 1978; Okoh et al., 1987). Apparently the consumption of sorghum sprouts or products made from them may be hazardous because of its high cyanide

content. Hydrocyanic acid (also called prussic acid) is a highly toxic substance, 300ppm being a lethal dose for adults, resulting in death within a few minutes. In small doses, the cyanide is detoxified to thiocyanate by the enzyme, rhodanese which makes use of sulphur amino acids as the sulphur donors, thereby making methionine the second limiting amino acid in sorghum-based diets.

The purpose of this study is to -

1. Undertake the proximate analysis of the sorghum grains used in the various diets in order to determine the effect of malting on the chemical composition of sorghum;
2. to determine the amounts of thiocyanate and cyanide (free and bound) in the gastrointestinal tract (wall and contents) plasma, faeces and urine in order to establish the metabolic route of dhurrin and its metabolites, and the extent of its toxicity to rabbits,

3. MATERIALS AND METHOD

Animal Experiment

In this experiment, twelve rabbits were used and feeding lasted for five weeks. Three-tier hutches were used in a roofed house with three treatments for four replications (consisting of one rabbit per replicate)
The rabbits were about 9 weeks of age with an average weight of about 1.06kg.

The three diets used (Table 9) were isonitrogenous (about 18% crude protein) and isocaloric (about 2,900 Kcal/kg). Other nutrients viz. ether extract, calcium, phosphorus, lysine, methionine : cystine were also of about the same levels in the threee diets.

The slight variations in the levels of molasses, soyabean meal and wheat offals was as a result of slight adjustments in the process of balancing the diets.
Sorghum inclusion rate was 32% in all diets. The difference between the diets in this respect was that diet I contained unmalted sorghum, diet II contained whole malted sorghum, while diet III contained malted sorghum but with roots and shoots removed. The differences among the diets was

to form the basis of comparing the rabbits fed the three diets, as shewn on page 85.

The weights of rabbits and feed consumed were determined as reported in the first animal experiment.

Urine samples were taken in the third and fifth weeks of the experiment. To collect urine, two methods were used. In one method, very soft cellophane bags were normally tied round the abdominal region of the individual rabbits. As soon as they passed urine, this was emptied in fear of the rabbit damaging the cellophane which will automatically empty its contents. The second method involved the use of plastic pans, about 45cm by 30cm by 8cm in length, width and depth respectively. The plastic pans were usually placed under each rabbits chamber. And since the hutches were of slatted wire types, if the rabbit urinated from above it, the urine was adequately collected, albeit sometimes with faecal contamination. This method proved the better method of urine collection.

There was no problem in faecal collection since faeces were dropped on the upper cage chambers (and therefore, easily collected) or on the floor. In collecting faeces, those that

Table 9: Ingredient and nutrient composition of the three experimental diets.

Ingredients	Diet I (With unmalted sorghum)	Diet II (With whole-malted sorghum)	Diet III (With malted sorghum, but roots and shoots removed)
Wheat offals	43.25	44.25	43.25
Sorghum	32	32	32
Soybean meal	19	18	21
Salt	0.50	0.50	0.50
Limestone	1.00	1.00	1.00
Molasses	4.00	4.00	2.00
Vit./Min. Mix.	0.25	0.25	0.25
Total	100	100	100
Analysis of nutrients:			
Crude protein (%)	17.84	17.85	17.20
DE (Kcal/kg)	2800	2850	2770
Crude fibre (%)	3.06	6.80	8.0
Ether extract (%)	3.11	3.00	3.20
Calcium (%)	0.50	0.55	0.49
Phosphorus (%)	0.30	0.76	0.81
Lysine (%)	ND	ND	ND
Methionine + Cystine	ND	ND	ND

ND = Not determined (because of lack of analyzer).

appeared likely to have been contaminated with urine or cecotrophs were avoided. Generally, faeces collection was in the fifth week. All samples taken were labelled and stored in refrigerators at temperatures below $0^{o}C$.

At the end of the fifth week, three rabbits from each treatment (nine rabbits in all) were randomly removed and slaughtered at the Meat Laboratory.

From the data collected, daily and cummulative weight gain and feed consumption rate were determined and feed efficiency (kg feed/kg gain) computed from it.

Proximate analysis of grain sorghum

Proximate analysis of grain sorghum was done in order to determine the effect of malting on the chemical composition of the grain. The cyanide levels in the three grain sorghum types were also determined as per procedures described from section 3.5.

Extraction of cyanogenic glucoside from sorghum

Exactly 0.1g of each ground sample of the root, shoot and whole seedling, and also 1g of the seed were weighed and extracted with 4 ml portions of 0.1m sodium phosphate buffer, pH 6.8. The extract was then stored in the freezer untill analyzed. This procedure ensured complete extraction of the cyanogenic glucoside present in the samples.

Extraction of bound cyanide, free cyanide and thiocyanate from animal tissues

Each tissue to be analyzed was cut into small pieces with a pair of scissors and homogenized. A known weight of the

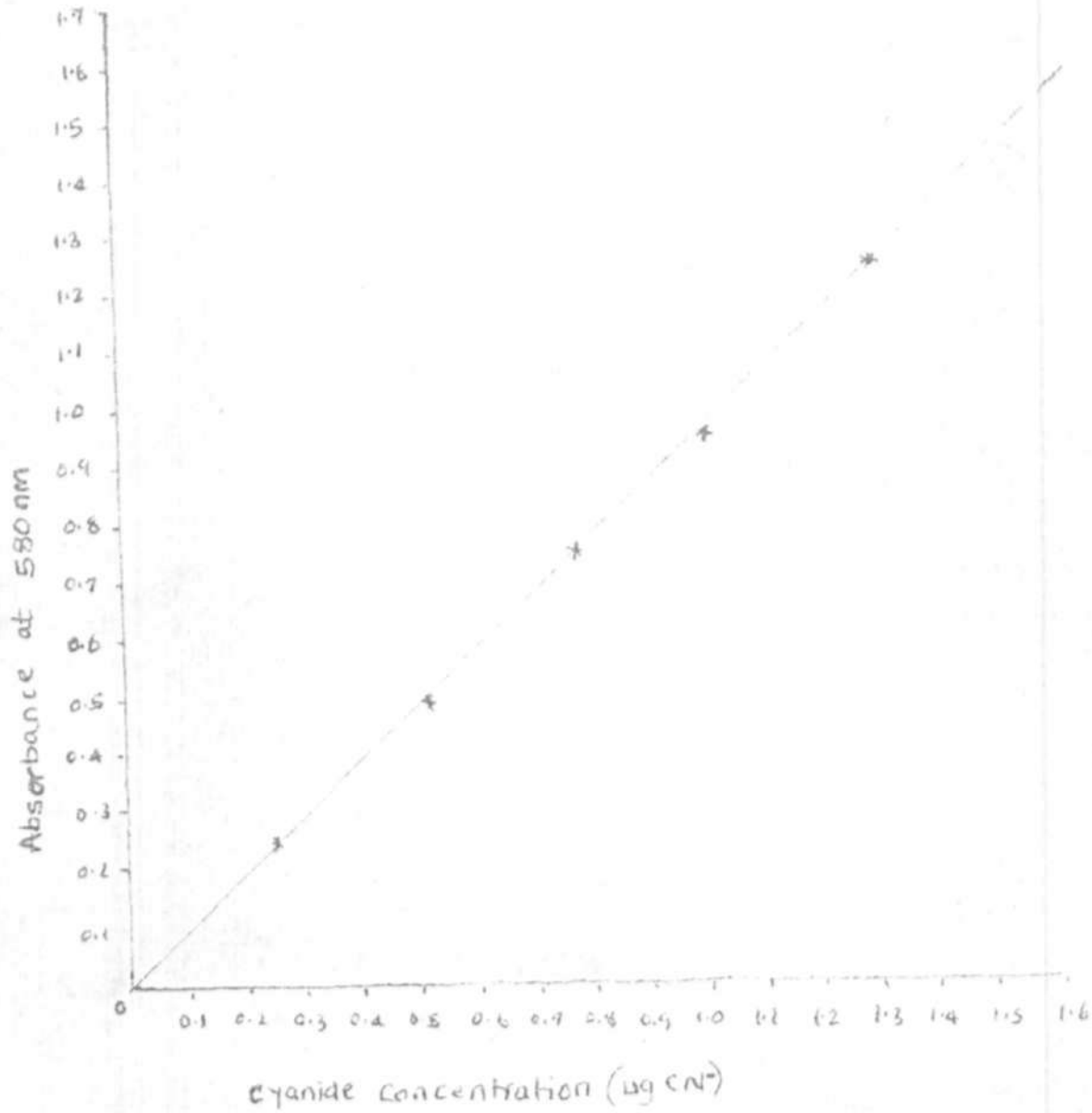
homogenate was then extracted thrice with 5 ml portion of 0.1M phosphate buffer pH 6.8. After each extraction, the homogenate was centrifuged, and the supernatant collected in small containers. The tissue contents (e.g. stomach, small intestine and large intestine contents) and faeces were also treated in the same manner as the tissues except that cutting with a pair of scissors was omitted. All sample extracts were kept frozen untill required for analysis.

Construction of a standard curve for cyanide assay using the Konig reaction (Lunquist et al., 1985).

A stock solution was prepared by dissolving 3.26g of KCN in distilled water and diluting to 100 ml. A working standard was prepared freshly as needed by diluting stock solution 500 fold with distilled water. Test-tubes containing 0.2 to 1.3 ug of cyanide were set up in duplicate. The volume of the working standard in each test tube was made up to 4 ml with 0.1 mole/L NaOH.

To each test tube was added 0.4 ml of 2 mole/L acetic acid and the mixture vortex mixed briefly. Sodium hypochlorite (0.1ml of 5 m mole/L) was added and vortex-mixed again. Within 1 minute, 0.5ml of barbituric acid - pyridine reagent was added and mixed. Absorbance of the resulting solution was read at 580mm in a 1cm cuvet between 5 and 15 minutes later. A blank determination was usually performed. The standard cyanide cruve produced from the spectronic readings is presented in Figure 8.

Figure 9: Standard curve for the estimation of cyanide by the Konig reaction.



Construction of a standard curve for thiocyanate assay using the method of Lundquist et al. 1979

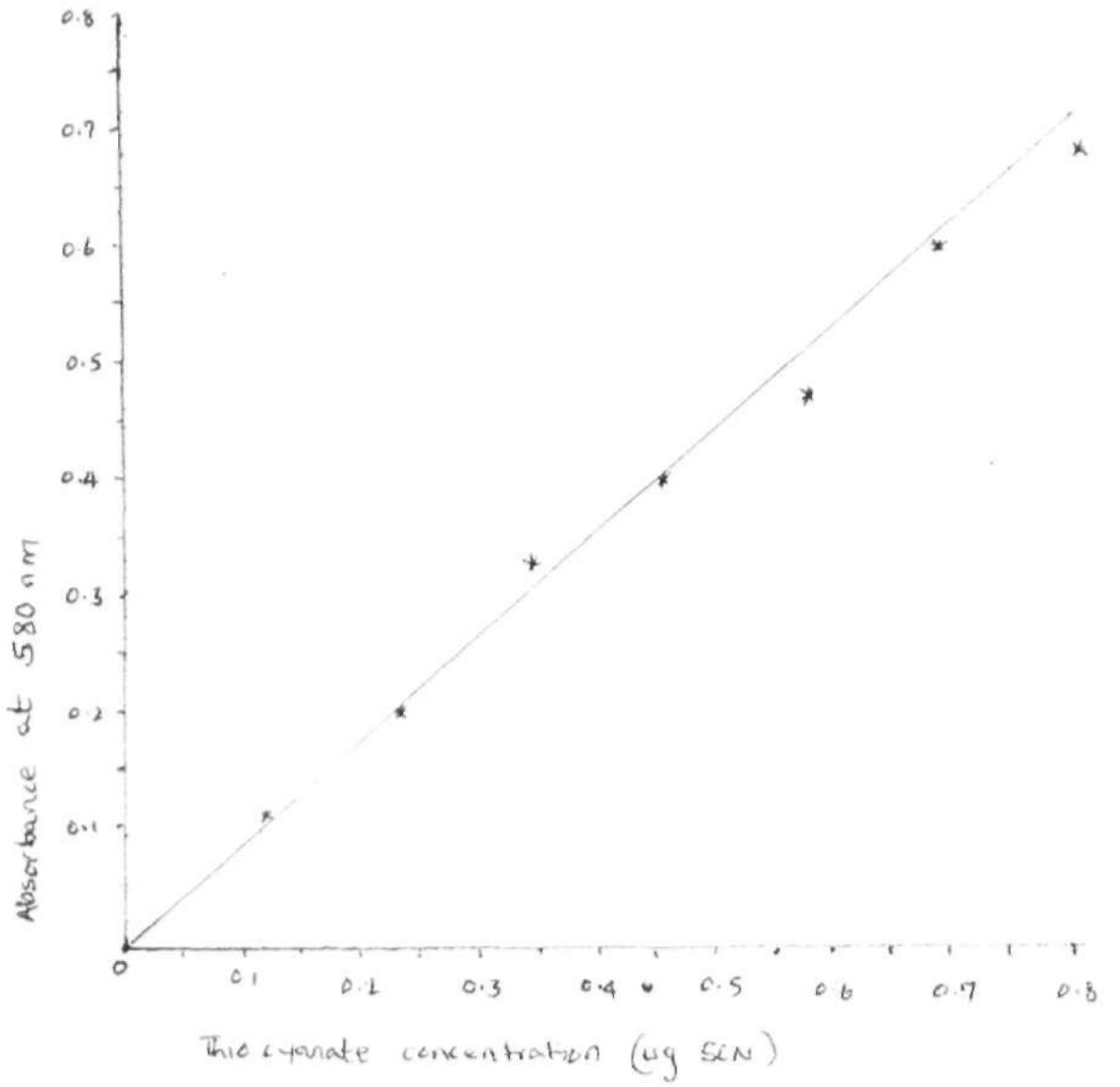
A standard potassium thiocyanate solution was prepared by dissolving 9.72g in 1 litre of distilled water. A working standard was prepared freshly as needed by diluting stock solution 500-fold with distilled water. From this standard solution, a series of test tubes containing from 0.1 to 0.7 ml of the potassium thiocyanate solution was set up. The volume of each was made up to 0.7 ml with distilled water. To each of the standard was added 3.3 ml of sodium perchlorate solution giving a total volume of 4.0 ml. Acetic acid (0.2 ml of 0.5M solution) was added to each test tube and vortex mixed. The sodium hypochlorite (0.1 ml of 50m mole/L solution) was added and vortex-mixed again. Within one minute, 0.5ml of barbituric acid-pyridine reagent was added and vortex-mixed. Absorbance of the resulting solution was read at 540 nm in a 1cm cuvet between 5 and 15 minutes later. A blank determination was also performed using 4 ml of perchloric acid. The standard thiocyanate curve produced from the appropriate readings is presented in Figure 10.

Assay of thiocyanate in tissue extracts, urine and plasma

Resin Preparation

Amberlit A 21 mesh was ground in a Techator model 1090 mill using the finest adjustment. It was washed with water and suspended in hydrochloric acid (1 mole/L). The resin

Figure10: Standard curve for thiocyanate estimatimation by the Lundquist method:



was filtered on a suction filter and washed with water untill the pH of the washings was 4.5 or greater. It was then suspended in water and left to stand for at least 15 minutes. The water decanted off and the resin resuspended in an equal volume of sodium hydroxide (1 mole/L). After 15 minutes the resin was washed with water untill the washings attained a neutral pH. It was then ready for use.

Column packing and preparing

The chromatographic columns were carefully packed with the prepared resin and corked from below to retain water above the column.

For extracts from CIP compartments, 5ml of extract was mixed with 0.5ml of 1% KOH and added to column. This was washed once with 5ml of 0.1M Hcl (to remove CN^-). Thiocyanate (SCN) was exluted from column with 8ml sodium perchlorate ($NaclO_4$) solution into a test tube.

For urine, 0.3ml of sample was added to 5.0ml of 0.1M NaOH and applied to column. In the case of plasma, 0.5ml was added to 5.0ml of 0.1M NaOH and applied to column. In each case, the column was washed once with 5ml distilled water and then twice with 5ml of 0.1M Hcl. Thiocyanate was then eluted from column with 8ml sodium perchlorate into test tube.

To 4ml of the eluate was added 0.2ml of acetic acid (0.5M) and votex-mixed. 0.1ml of 50m mole/L sodium chlorite (NaclO) solution was added and votex-mixed again. Within one

minute, 0.5 ml of barbituric acid-pyridine reagent was added and votex-mixed. Absorbance was determined in a spectronic 20 at 580nm within 5 to 15 minutes against distilled water.

Assay of free and bound cyanide in sorghum or tissue extracts, urine and plasma

The methods for the determination of free, bound and total cyanide via nitrogen aeration (Dixon and Rickards, 1952 and Lundquist et al. 1985) is described. Free cyanide was determined directly by the aeration procedure (described later). To measure total cyanide, the bound cyanide was first hydrolyzed by means of NaOH and the resulting total free cyanide was assayed. Bound cyanide was determined by simple difference between total and free cyanide.

For practical purpose, the method described by Cooke (1978) and Olugboji (1987) was employed as follows:

Aeration procedure for free cyanide

1 ml of sample or extract was taken, 10 ml of distilled water added(to increase volume). To this was added 5 drops of antifoaming agent, then 2 drops of methyl orange. This was acified by the addition of 1ml of 0.5M H_2SO_4 (if colour turned purple, then acid was considered adequate). This was corked immediately and aerated for 45 minutes (using nitrogen compressed in a pressure cylinder and regulated via a nossle). The aerate (cyanide) now separated from the sample was trapped in 4 mls of 0.1M NaOH in the smaller aeration tubes. After the aeration, the stoppers from the NaOH traps were removed with care to

avoid the sucking back of any NaOH from the inlet tube into the trap.

To assay, 4 ml of aliquot (or 2ml and made up to 4ml with 0.1M NaOH) was taken. 0.4ml of 2M acetic acid was added and votex-mixed. Then 0.1ml of 50m mole/L NaOcl was added and votex mixed. Within one minute, 0.5ml of barbituric acid-pyridine reagent was added and votex-mixed. With 5 to 15 minutes, absorbance on spectronic 20 was read at 580nm. The cyanide concentration of the sample was read from the cyanide standard curve (Figure 1).

Total cyanide determination

There was initial hydrolysis. Exactly 1ml of sample extract was pipetted into the larger aeration tubes. This was followed by the addition of 10ml of 0.1M NaOH solution and the solution was incubated for 30 minutes at room temperature to hydrolyze the cyanogenic glucoside. At the end of incubation, 5 drops of antifoaming agent was added, followed by 2 drops of methyl orange indicator (to confirm acidity). Then 1ml of 0.5M H_2SO_4 was added (and solution turned purple). It corked immediately and aerated for 45 minutes, trapping the cyanide in 4ml of 0.1M NaOH in the lower (smaller) tubes. This was assayed as in free cyanide procedure already described.

Proximate composition and cyanide content of malted and unmalted sorghum grain

The proximate composition of both malted and unmalted sorghum grain is shown in Table 10. Crude protein of grain increased from 9.38% to 10.27% on dry matter basis by the fourth day of germination. This agrees with the work of Wu and Wall (1980) who reported that the six-day sprouted grain had higher protein compared with the starting material. Kubiczek et al. (1984) also reported 3.7% protein increment during four-days' malting of SK 5912 sorghum variety. When the roots and shoots were removed, the protein content of the malted grain dropped from 10.27 to 6.25% (on dry matter basis).

Total and free cyanide increased during the four-day malting from 22 to 963 ppm and 22 to 168 ppm respectively. This increase in cyanide as a result of malting is in agreement with the findings of Conn (1969). However, removal of roots and shoots resulted in the loss of all the cyanide formed during malting (Table 10).

Average daily feed consumption, weight gain and feed efficiency

Results were collected at the end of the experiment showing average daily feed consumed (g) averaged daily weight gain (g) and efficiency of feed conversion. The mean values for all treatments are presented in table 11.

Average daily feed consumption was highest in treatment I and lowest in treatment II. The variance ratio showed that the difference between the feed consumption among the animals (replication) is statistically significant ($P<0.05$). The feed consumption rate between treatments and weeks was, however statistically insignificant ($P>0.05$).

Table 10 : Proximate composition and cyanide content of whole malted, unmalted and malted sorghum without shoot and roots

Component	Malted sorghum (with roots and shoots)	Unmalted sorghum	Malted sundried sorghum without roots and shoots.
*DE (Kcal/kg)	3454.70	3421.30	3406.26
Crude protein (%)	10.27	9.38	6.25
Dry matter (%)	92.33	92.07	ND
Ash (%)	1.29	1.45	ND
Ether extract (%)	1.90	2.92	ND
Crude fibre (%)	7.49	7.41	7.90
Total cyanide (ppm)	963	44	ND
Bound cyanide (ppm)	795	22	23
Free cyanide (ppm)	168	22	ND

* = Conversion as per Oyenuga (1968) and Philips (1977)

ND = Not determined (samples tempered with prior to analysis)

Table 12 : Effect of malted sorghum on average daily feed intake (g), average daily gain (g) and feed conversion efficiency .

Treatment	Average daily feed intake (g)	Average daily weight gain (g)	Mean feed conversion efficiency
Unmalted	66.07	13.41	8.85
Malted (with roots and shoots)	63.46	15.35	5.15
Malted (shoots and roots removed)	64.99	13.19	7.14

Average daily weight gain was highest in treatment II and lowest in treatment III. The analysis of variance showed that the difference between the weight gain among the animals (replication) was high but not statistically significant (P/0.05). The difference between weeks and between treatments were farless significant.

Efficiency of feed conversion was lowest (therefore best) in treatment II followed by treatments III and I respectively.

The difference between treatments, weeks and replication were not statistically significant.

Cyanide and thiocyanate values

The heparin (anti coagulant) used in storing the blood plasma prior to assay was not effective. As such the assay of both cyanide and thiocyanate of the blood plasma was not possible since there was some degree of coagulation previously.

The concentration of both cyanide and thiocyanate in the gastrointestinal tract (GIT) of the rabbits were found to be in traces (generally lower than 1 ppm). But even in that situation, the values for rabbits on diet II (i.e. treatment II which had whole malted sorghum) always had higher cyanide and thiocyanate values. The distribution pattern of cyanide and thiocyanate in the wall and contents of the GIT was not distinct, as a result of which no conclusion could be arrived at as to which of the two had higher concentration of either cyanide or thiocyanate than the other.

The thiocyanate values obtained in the faeces of the rabbits are presented in Table 13. It shows that thiocyanate was also found in traces in the faeces. Treatment differences were not statistically significant. Cyanide was, however, hardly detectable in the same faeces samples.

Urine thiocyanate values were more appreciable (Table 13). The mean thiocyanate value for treatment II in first urine collection (14.798 ug/ml) was clearly higher than that for treatments I and III which were 1.280 and 2.478 ug/ml respectively. The difference was statistically significant. The second urine collection generally had higher (slightly more than double on the average) thiocyanate values than the first urine collection. The difference between treatments was statistically significant ($P<0.05$); treatment II having mean value of 34.768 ug/ml as against 3.097 and 3.554 ug/ml respectively for treatments I and III.

Table 13 : Mean faecal and Urinary thiocyanate levels of the rabbits

Treatment	Faecal SCN[a] (ug/g)	SCN (ug/ml)[a] in first urine	SCN (ug/ml)[a] in second urine
Unmalted sorghum	0.722	1.280	3.097
Whole malted sorghum (with roots and shoots)	1.320	14.795	38.768
Malted sorghum, roots and shoots removed	0.803	[illegible]	3.554

a = Statistically significant ($P < 0.05$).

Calculated cyanide intake by the rabbits

Based on the levels of cyanide found in the grain sorghum (malted and unmalted) which were fed to the rabbits, expected rates of consumption and therefore levels to be found in their bodies were calculated. The procedure and results are in Table 14 and Appendix K.

Table 14: Cyanide intake by the rabbits

Treatment	% body total CN consumed $X10^{-6}$	% body free CN consumed $X10^{-6}$
I	0.62	0.31
II	33.90	5.90

5. DISCUSSION

Malting of sorghum

The Wu and Wall (1980) technique of malting was found to be a good method. Its fungal control ability was equally good since very few mouldy grains were found even under large scale malting.

Proximate composition and cyanide content of the malted sorghum grain

Malting proved to slightly increase DE (digestible energy) in sorghum grains (Table 12). The removal of roots and shoots from whole malted sorghum did not only decrease DE but also lowered crude protein level from 10.27 to 6.25%. Whole matled sorghum had higher protein level (10.27%) than unmalted sorghum (9.38%). Fat content, although not changing significantly, decreased slightly leading to the conclusion that portions of it could have been used to augment the energy requirements of the germinating grain. There was also no much change in crude fibre levels. The slightly higher level in malted grains having roots and shoots removed reflects an expected increase in fibre relative to digestible carbohydrates that were depleted as a result of removal of the roots and shoots. On the average therefore, malting seemed to have improved the nutritive status of the sorghum grains used.

The amounts of total and bound cyanide found in the malted grain which were 963 ppm and 795 ppm respectively are close to the findings of Parasiuk and Bill. (1980) which was 693 ppm HCN. The increase in total and free cyanide during

malting from 22 to 963 ppm and 22 to 168 ppm respectively is in line with the findings of Conn (1969) who reported that on germination, cyanide content of sorghum increased. The results further show that bound cyanide (dhurrin) in this case constitutes most (about 83%) of the total cyanide in sorghum. The toxicity of malted sorghum therefore, largely depends on the rate of metabolic hydrolysis of dhurrin (Okoh, 1978). Orinarily, if fed alone, 613 ppm HCN is above lethal dosage. Besides the hydrolysis factor, danger was further lessened during feeding as the rate of sorghum inclusion was pegged at 32% of the three diets. The implications of these are further considered in relation to the levels of cyanide and thiocyanate found as physiological indices. When roots and shoots were removed, the bound cyanide levels in the grain was found to be about the same with the unmalted grain. This shows that the cyanide is usually concentrated in the meristematic regions.

Cyanide consumption

From the computations in Table 14, it is seen that both total and free cyanide values are very low. The highest amount of total cyanide consumed by a rabbit (Appendix X) in treatment II (0.000095% body weight) is comparatively too low to be effective if this is placed against toxic values of 0.2% (2000ppm) in young sorghum leaves reported by Conn (1969). Cummulative consumption over five weeks gave total cyanide for the above animal (35 x 0.000095) as 0.0033% Cyanide

level of 0.0033% body weight for the animal (which is equivalent of 33 ppm) is quite harmless. The toxicity in this experiment was further lessened because of the metabolic hydrolysis factor mentioned earlier (bound cyanide constitutes 83% total cyanide). Secondly, it has been proved (Okoh, 1978) that under chronic feeding of cyanide (especially at relatively harmless level as in our own case), no cyanide may be demonstrated in tissues by known chemical methods. This, according to Okoh (1978) is because cyanide is rapidly metabolized in animals largely to thiocyanate (SCN^-).

At an intake rate of 0.5% to 0.8% of body weight, tapioca leaf containing 7.6mg of HCN per 100g of material did not bring about any adverse effect (Ranjhan, 1980). The work of Omole and Ashiett (1976) on feeding cassava diets to rabbits showed that the rate of gain decreased with increasing levels of cassava in the ration, although this decrease was not significant until the ration contained 45% cassava root meal. Hence, the level of inclusion of sorghum in our diet could hardly have a significant effect on the animals.

Cyanide and thiocyanate levels in physiological indices of the rabbits

Since the assay of blood plasma for both cyanide and thiocyanate was not possible, this will need to be done in order to present a more complete picture of the physiological indices.

That the concentration of both cyanide and thiocyanate were found only in traces in the GIT may largely be attributed to ineffective extraction procedure prior to assay. This is why there was no clear trend especially with respect to the higher thiocyanate concentration in the stomach as a result of GIT circulation as reported by Okoh and Pitt (1982). The results (Table 4) nevertheless showed that malting has effect on the cyanide and thuocyanate levels of urine, and to some extent faeces, of animals consuming them. This is why treatment II maintained higher levels of the two in both urine, faeces and GIT.

The little amounts of thiocyanate found in faeces likely to be as a result of urine contamination of faeces during feeding. The relative absence of dhurrin in the faeces implies that any unhydrolyzed dhurrin was completely absorbed in the GIT. Also, the absence of thiocyanate in faeces even where there were traces in the GIT coupled with the larger amounts of thiocyanate in the urine shows there was systematic reabsorption of thiocyanate by the kidneys. This is said to be a continuous metabolic process (Conn, 1969). Furthermore, urine has been confirmed (Okoh and Pitt, 1982, Okoh, 1983) as the major route of elimination of thiocyanate in the body. Our results reflected this.

The cummulative effect of feeding malted sorghum reflected in the systematic increase in thiocyanate levels in the urine collected. For while first, urine collection (Table 13) had 14.796 ug/ml of SCN^- on the average in treatment II, it went to as high as 34.768 ug/ml in the same treatment during second urine collection which came two weeks after the first. As usual, treatment II always had the highest values. This means the higher and longer the intake of cyanogens, the more the amount of thiocyanate accumulated in the body.

6. CONCLUSION

The process of malting bestowed nutritional benefits to the sorghum grains which were effectively utilized by the rabbits. This reflected in better growth rate in rabbits fed malted sorghum-based diets.

But while malting improved the nutritive status of the sorghum, it also increased tremendously the levels of dhurrin in the grains, total and free cyanide increasing from 22 to 963 ppm and 22 to 168 ppm respectively. Much as these values were comparable to reported lethal values of 613ppm (Parasiuk and Bills, 1984) if fed alone, the rate of inclusion in our diets (32%) was aimed at lessening the possible toxicity. In addition, the fact that 83% of the total cyanide values were bound cyandie and since cyanide toxicity largely depends on the rate of metabolic hydrolysis of dhurrin, the effective intake of cyanide was rendered virtually harmless to the rabbits.

Calculated levels of cyanide consumed by the rabbits showed that the highest amount consumed was 0.0095% of body weight which is far lower than toxic values of 0.2% reported by Conn (1969) in young sorghum leaves. Since even at intake rate of 0.5 to 0.8% body weight, tapioca leaf containing 7.6 ug of HCN per 100g of material did not bring about any adverse effect (Ranjhan, 1980), it is recommended that inorganic cyanide be incorporated at graded levels in addition to the cyanogenic glucoside for any meaningful cyanide toxicity experiment.

The absence of thiocyanate in the faeces even when there were traces in the GIT shows that there was systematic reabsorption of thiocyanate by the kidney, which is said to be a continuous metabolic process (Conn, 1969). This leads to the high concentration of thiocyanate in the urine as reflected in our results. This agrees with the findings of Okoh (1983) which reported urine as the major route of thiocyanate removal from the body.

The increase in the rate of thiocyanate removal through urine with time proves that the higher and longer the intake of cyanogens, the more the amount of thiocyanate formed from cyanide.

GENERAL SUMMARY AND CONCLUSION

The sorghum malting technique of Wu and Wall (1980) which was adopted in the experiments proved an effective method for germination of sorghum even on commercial basis. Its ability to keep low the rate of mould formation even on large scale basis recommends it for use by breweries and other industrial concerns.

Although cyanide content of the malted sorghum were found to be quite high (963 ppm total cyanide), its deleterious effects were apparently avoided by ensuring that the maximum level of sorghum inclusion (32%) was below the lowest toxicity threshold of about 45% inclusion rate. Since sorghum had a much lower cyanide value (about 963 ppm) in this case than the experimented cassava (about 2,400 ppm), the dangers were much less. Again, bound cyanide constituted about 83% of the total cyanide in the sorghum, and since toxicity of cyanogenic glucosides largely depends on the rate of metabolic hydrolysis in the animal (Okoh, 1978), the real dangers of toxicity in our experiments were further reduced.

The fact that malting did not affect feed intake in both experiments is a desirable finding. Thus, even at the inclusion rate of 32%, malted sorghum did not affect rate of feed intake. Malting, however, improved both mean daily weight gain and feed efficiency of rabbits. The best results of 15.61g/day and 3.35 for mean weight gain and feed efficiency respectively compare favourably with results of 15.80g/day and 3.30 respectively

elsewhere (Harris et al., 1983).

The experiments have confirmed that rabbits utilized the nutritional benefits of malted sorghum. In particular the benefits of increased protein and amino acid utilization as well as decreased tannin content were all combined. It is therefore recommended that sorghum be malted before being fed to domestic animals. This practice will be very suitable for backyard farmers but also possible on large scale production where malting is convenient. Since fermentation, especially ensiling, is an effective cyanide detoxification technique (Doggett, 1970), the possibility of producing germinated and subsequently-ensiled sorghum may be further investigated. The nutritional and economic benefits of such a practice must be considered side by side.

The roots and shoots were found to be the 'custodians' of the benefits of malting sorghum. Hence their removal lowered its nutritional quality to about that of the unmalted grain, as overall performance of the rabbits suggested. Although cyanide is concentrated in the roots and shoots (Ikediobi et al. 1987), considering the rather harmless levels of sorghum inclusion in our experiments, they are better left intact (since removal of roots and shoots drastically reduced the crude protein level from 10.27% to 6.25%).

Calculated levels of cyanide consumed by the rabbits showed that the highest amount consumed was 0.009% of body weight (Appendix X) which is far lower than toxic values of 0.2% reported by Conn (1969) in young sorghum leaves. Since even at intake rate of 0.5 to 0.8% body weight, tapioca leaf containing 7.6mg of HCN per 100g of material did not bring about any adverse effect (Ranjhan, 1980).

The absence of thiocyanate in the faeces even when there were traces in the GIT showed that there was systematic reabsorption of thiocyanate by the kidney, which is said to be a continuous metabolic process (Conn, 1969). This leads to tye high concentration of thiocyanate in the urine as relfected in the results. This agrees with the findings of (Okoh and Pitt 1982, Okoh, 1983) which reported urine as the major route of thiocyanate removal from the body.

The increase in the rate of thiocyanate removal through urine with time proves that the higher and longer the intake of cyanogesn the more the detoxification rate of cyanide to produce thiocyanate.

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APPENDICES:

APPENDIX I: THIOCYANATE STANDARD CURVE PREPARATION

Potassium thiocyanate (KSCN) standard solution is used to prepare a thiocyanate curve from a graph plot of photospectrometric absorbance (optical density) against concentration of the thiocyanate.

1M KSCN = 97.2g/L

∴ 0.1M = 9.72g/L (standard stock solution)

Dilute 1000 x = 0.1mM/L

If it is 500 x dilution, = 0.2mM

i.e. $\frac{9.92}{500}$ = 0.019444g/L

= 0.00001944g/ml

= 19.44 mg/ml

= 19.44mgKSCN/ml

Mol. wt. of SCN = 58g

To obtain 200mg SCN:

1M SCN = 58g/L

1mM SCN = 58Mg/L

∴ 200mM/L SCN = 11600Mg/L = 11.6Mg/ml.

If 58g = 11.6

∴ 97.2 = $\frac{11.6}{58}$ x 97.2 = 19.44Mg/ml

1ml of working standard contains 11.6Mg SCN.

Dilute 10 times to obtain the desired and measurable absorbance in the spectrophotometer

APPENDIX II: CYANIDE STANDARD CURVE PREPARATION:

Potassium cyanide (KCN) standard working solution is used to prepare a cyanide curve from a graph plot of optical density (O.D.) against concentration of the cyanide.

Standard Stock Solution = 0.5M KCN

Working Standard Solution = 1mMKCN

10 times dilution = 0.1mM KCN

1mole KCN = 65.2g

∴ mMole = 65.2 mg

∴ 0.1 mMole = 6.52mg/L

0.1mMole = 6.52Mg/ml.

But CN = 26g

If 65.2 = 6.52mg/ml

(Mol. wt. of CN = 26)

∴ $26 = \frac{6.52}{65.2} \times 26$ = 2.6Mg CN/ml. in 0.1mM KCN.

i.e. 0.1mM Solution KCN contains 2.6Mg CN/ml.

Since 1ml contain 2.6MgCN

∴ 0.1ml ——→ 0.26

0.2ml ——→ 0.52

0.3ml ——→ 0.78

0.4ml ——→ 1.04

0.5ml ——→ 1.30

0.6ml ——→ 1.56

0.7ml ——→ 1.82

APPENDIX III: Cyanide and thiocyanate standard working solution volume, absorbance and concentration as determined

Volume of standard working solution(mls)	Absorbance at 580 nm	SCN^- concentration (Mg SCN^-)
0.1	0.110	0.116
0.2	0.200	0.232
0.3	0.330	0.348
0.4	0.400	0.464
0.5	0.470	0.580
0.6	0.600	0.696
0.7	0.680	0.812

Volume of standard working solution(mls)	Absorbance at 580nm	CN concentration (Mg CN)
0.1	0.244	0.260
0.2	0.490	0.520
0.3	0.755	0.780
0.4	0.945	1.040
0.5	1.250	1.300

APPENDIX IV: How to Calculate thiocyanate values from absorbance or optical density (O.D) readings:

1. For GIT and faecal extracts:

x (O.D) x 2 = Y

To get per ml concentration = Y/S = Z

To get per gm = Z x 7.5

NB: X is multiplied by 2 because only 4mls out of total 8mls evaluate is used. Y is divided by 5 because 5mls of extract is eluted through the column Z is multiplied by 7.5 because 15 Mls extract was produced from 2gm sample ($\frac{15}{2}$ = 7.5).

2. For Urine

X x 2 = Y

To get per ml concentration = Y x 3.33 = Z

NB: Y is multiplied by 3.33 because 0.3mls of urine was eluted ($\frac{1}{0.3}$ = 3.33)

3. For Plasma

X x 2 = Y

To get per ml concentration = Y x 2 = Z

NB: Y is multiplied by 2 because 0.5mls of plasma was eluted.

($\frac{1}{0.5}$ = 2)

In some situations where initial colouration was too thick for the optimum strength of the photospectrometer, the eluate was diluted with perchlorate in order to get good readings. Two examples are given below with their methods of calculating their concentration.

i. 0.5mls eluate + 3.5mls perchlorate) = X x 16 = Y, Y x 3.33 = Z

NB: X is multiplied by 16 because only 0.5mls of eluate was used ($\frac{8}{0.5}$ = 16)

ii. 1ml eluate) + 3mls perchlorate) = X x 8 = Y, Y x 3.33 = Z

NB: X is multiplied by 8 because 1ml eluate was used out of total 8mls collected.

How to Calculate Cyanide values:

Since only 1ml is used at a time, therefore per ml values are the same as O.D. values. To get per gm values = X 7.5.

APPENDIX V : The effect of malted sorghum on the average daily feed intake of rabbits (g/rabbit/day)

Trt.	Replication	Week 1	Week 2	Week 3	Week	Week 5	$\bar{X}$
I	1	50.71	72.86	74.14	63.43	70.71	
	2	46.29	50.86	50.00	58.57	60.14	66.07
	3	52.14	75.71	66.86	75.00	72.86	
	4	62.86	72.14	72.29	73.57	80.29	
II	1	85.00	80.71	61.14	77.71	84.71	
	2	55.14	71.43	68.71	85.29	77.71	63.46
	3	44.28	52.86	38.86	48.57	44.14	
	4	61.71	66.43	39.86	59.43	65.57	
III	1	65.43	60.71	50.43	65.86	70.43	
	2	70.86	64.29	66.29	67.43	66.00	
	3	51.57	57.14	57.57	53.71	65.43	64.99
	4	77.00	82.86	74.00	71.86	60.86	
$\bar{X}$		60.25	67.33	60.01	68.37	68.24	

Source of variation	D.F	MS	F
Replication	3	489.482	3.787*
Treatments	2	34.3418	0.266
Weeks	4	223.786	1.731
Trs X Weeks	8	110.264	0.853
Residual	42	129.262	
Total	59		

APPENDIX VI : Effect of malted sorghum on the average daily weight gain of rabbits (g/rabbit/day)

Trt.	Replication	Week 1	Week 2	Week 3	Week 4	Week 5	$\bar{X}$
I	1	23.59	8.58	7.15	5.0	1[illegible].72	
	2	17.55	7.52	12.92	22.56	[illegible].15	13.41
	3	16.05	16.05	11.33	4.90	9.79	
	4	5.69	14.17	15.56	29.25	16.90	
II	1	29.98	7.14	15.70	17.42	14.00	
	2	4.83	11.21	19.23	13.62	15.07	15.35
	3	16.82	6.72	11.77	5.05	20.61	
	4	13.89	22.22	18.20	18.46	17.00	
III	1	22.86	2.86	7.14	9.57	10.42	
	2	13.46	9.67	16.55	3.80	8.96	13.19
	3	17.61	16.05	4.02	6.02	9.83	
	4	38.20	15.67	13.43	25.61	12.09	
$\bar{X}$		18.38	11.49	12.75	13.44	13.86	

Source of variation	DF	MS	F
Replications	3	133.846	2.82
Treatments	2	28.144	0.593
Weeks	4	82.100	1.73
Trt. X Weeks	8	42.077	0.887
Residual	42	47.461	
Total	59		

Appendix VII : Effect of malted sorghum on the average efficiency of feed conversion (g feed/g weight gain)

Trt.	Replication	Week 1	Week 2	Week 3	Week 4	Week 5	$\bar{X}$
I	1	2.15	8.50	10.38	12.67	4.50	8.85
	2	2.64	6.75	3.87	2.59	7.38	
	3	3.25	4.71	5.90	15.31	7.44	
	4	11.05	5.09	4.65	3.20	4.82	
II	1	2.84	11.35	3.90	4.48	6.07	5.15
	2	11.42	6.37	3.57	6.26	5.16	
	3	2.63	7.87	3.30	9.62	1.54	
	4	4.44	2.99	2.19	3.22	3.86	
III	1	2.86	21.23	7.06	6.88	6.75	7.14
	2	5.26	6.65	4.01	17.74	7.37	
	3	2.93	3.56	14.32	8.92	6.66	
	4	2.02	5.29	5.51	2.81	5.03	
	$\bar{X}$	4.46	7.53	9.89	7.81	5.55	

Source of variation	DF	MS	F
Replications	3	76.862	1.374
Treatments	2	68.273	1.221
Weeks	4	53.469	0.956
Trts. X Weeks	8	53.925	0.964
Residual	42	55.931	
Total	59		

APPENDIX VIII : Slaughter Records

Rabbit Code	Tag No	Breed	Sex	Live-weight (kg)	Fresh Carcass weight (kg)	Weight of head (kg)	Dressing %
T_1R_1	50	CROSS (Newzealand white/Dutch)	Male	1.380	0.656	0.140	47.54
T_1R_2	51	NewZealand White	Female	1.840	1.050	0.144	57.07
T_1R_3	52	CROSS (NewZealand white/Dutch)	Female	1.469	0.740	0.135	50.64
T_2R_1	46	NewZealand White	Female	1.600	0.785	0.140	49.31
		"	Male	1.615	0.819	0.176	50.71
T_2R_6	48	"	"	1.745	0.865	0.179	49.57
T_3R_1	49	NewZealand White	Male	1.490	0.747	0.140	50.13
T_3R_4	53	"	"	1.600	0.750	0.163	46.88
T_3R_6	54	"	"	1.400	0.678	0.130	48.43

APPENDIX IX : Weight of all rabbit GIT compartments (in grams)

Rabbit Code	GIT Compartment	Total	Contents	Wall
T_1R_1	Stomach	114.849	87.249	27.600
	Small intestine	62.511	31.827	30.684
	Cecum	94.679	85.571	9.108
	Large intestine	37.518	15.007	22.511
T_1R_2	Stomach	121.448	92.743	28.705
	Small intestine	64.900	33.476	31.424
	Cecum	97.550	88.739	8.811
	Large intestine	45.408	13.608	31.800
T_1R_3	Stomach	100.900	79.758	21.142
	Small intestine	59.045	28.565	30.480
	Cecum	98.56	88.364	10.196
	Large intestine	32.515	10.555	21.960
T_2R_1	Stomach	118.083	93.286	24.797
	Small intestine	54.716	23.708	31.008
	Cecum	92.008	82.448	9.560
	Large intestine	47.676	33.421	14.255
T_2R_2	Stomach	119.664	92.002	27.642
	Small intestine	60.892	31.492	29.400
	Cecum	91.333	82.430	8.903
	Large intestine	42.519	30.044	12.875
T_2R_4	Stomach	110.747	84.167	26.580
	Small intestine	61.876	29.936	31.940
	Cecum	92.266	83.224	9.042
	Large intestine	51.772	26.917	24.855
T_3R_1	Stomach	78.701	58.241	20.46
	Small intestine	52.375	26.711	25.664
	Cecum	80.040	70.435	9.605
	Large intestine	50.489	18.681	31.808
T_3R_4	Stomach	105.304	83.190	22.114
	Small intestine	54.778	25.956	28.822
	Cecum	81.616	74.060	7.550
	Large intestine	37.590	10.600	26.990
T_3R_3	Stomach	84.920	67.936	16.984
	Small intestine	50.051	25.388	24.663
	Cecum	71.744	63.944	7.800
	Large intestine	32.812	12.956	19.856

APPENDIX X : Calculated cyanide intake by the rabbits

Trt.	Repl.	Weight of rabbit (g)	Total feed consumed (g)	x0.32	Per day ÷ 35	Total CN consumed (g/animal) x 0.000044	% body total CN consumed ÷ body weight	% body free CN x 0.5
I	1	1,375	2322.95	743.34	21.24	0.00093	0.00000068	0.0000034
	2	1,468	1861,82	595.53	17.02	0.00075	0.00000050	0.00000025
	3	1,615	2397.99	767.36	21.92	0.00096	0.00000059	0.00000029
	4	1,555	2668.05	853.78	24.39	0.00107	0.00000069	0.00000035
						x0.000963		x0.175
II	1	1,630	2724.85	871.95	24.91	0.024	0.000013	0.0000023
	2	1,339	2507.96	802.55	22.93	0.022	0.000016	0.0000028
	3	1,195	1600.97	512.31	14.64	0.014	0.0000118	0.0000021
	4	1,900	2051.00	656.32	18.75	0.018	0.000095	0.000017

Appendix XI : Special Reagents and Chemicals

Sodium hypochlorite, 5 m Mole/L:

0.1 ml of NaclO (reagent no. 23039, BDH chemicals, Pode, England) was diluted to 10 ml with distilled water. This solution was prepared daily for cyanide assay.

Sodium hypochlorite, 50m Mole/L:

1.0ml of NaclO was diluted to 10 ml with distilled water. This solution was prepared daily for thiocyanate assay.

Barbituric acid - pyridine reagent:

6.0g of barbituric acid was dissolved in a mixture of 30ml of pyridine and 61ml of water. 6.0ml of concentrated hydrochloric acid was then added and mixed.

Formaldehyde:

The formaldehyde used to prevent mould growth during melting was obtained from Hopkin and William and Co., Chadwell Health, Essex, England.

Sodium phosphate buffer

0.1M sodium phosphate buffer, pH 6.8 was prepared by mixing 24.50 Ml of Na_2HPO_4 solution (3.582g of Na_2HPO_4 in 100 ml solution) and 25.5ml NaH_2PO_4 solution (1.56g of NaH_2PO_4 in 100 ml solution) and diluting to 100 ml with distilled water.

Amberlit A21 anion exchange resin.:

This is a product of Rohm and Hass Co Philadelphia, P.A.

Sodium perchlorate, 2 mole/L

281g of sodium perchlorate was diulted in one litre of distilled water to give 2 mole/L solution.

All samples analysed were obtained from experiments conducted in Section I.

Urine samples were taken in the third and fifth weeks of the experiment. To collect urine, two methods were used. In one method, very soft cellophane bags were normally tied round the abdominal region of the individual rabbits. As soon as they passed urine; this was emptied in fear of the rabbit damaging the cellophane which will automatically empty its contents. The second method involved the use of plastic pans, about 45cm by 30cm by 8cm in length, width and depth respectively. The plastic pans were usually placed under each rabbits chamber. And since the hutches were slatted wire types, if the rabbit urinated from above it, the urine was adequately collected, albeit sometimes with faecal contamination. This method proved the better method of urine collection.

There was no problem in faecal collection since faeces were dropped on the upper cage chambers (and easily collected) or on the floor. In collecting faeces, those that appeared likely to have been contaminated with urine or cecotrophs were avoided. Generally, faeces collection was in the fifth week.

BIOGRAPHY OF AUTHOR

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Bauchi State Min. of Animal and Forest Resources	1983/84	Animal Husbandry Officer/ Livestock Instructor, School of Agriculture and Animal Husbandry, Bauchi.
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