Studies on cassava production and preparing methods for utilization as feed

キャッサバの生産と飼料利用のための調製技術に関する研究

YIN YIN KYAWT

Science of Bioresource Production

The United Graduate School of Agricultural Sciences

Kagoshima University

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Contents

CHAPTER 1

General Introduction

Important components in animal feeds for maintenance, growth and reproduction are energy and protein. At present the scarcity of conventional protein and energy resources is largely responsible for the high price of livestock feed. The feed industry is faced with a number of challenges, not only regarding the availability of feed ingredients but also the ability to produce high quality products in a cost-effective manner. There is therefore need to reduce the competition between human being and livestock for the same feedstuffs by turning to unconventional feedstuffs in the short run while plant breeders work towards obtaining high yielding varieties of crop which will ensure adequate surplus and quality feed for livestock (Uchegbu, 2005). The poultry industry has also become the predominant source of protein in the diet of the population of most developed and developing countries. It had earlier been reported that future expansion and sustenance of poultry industry depend on availability of grains above that required for human consumption (Patrick and Schaible, 1980). Maize has been the most commonly used conventional energy source of plant origin in feed formulation in poultry. It is however, used as human food and for various industrial raw materials. Substantial efforts have been made, in the past few decades, to replace cereals with

other carbohydrate sources, such as sorghum (Rajasekher *et al*., 2000), lentils, cassava tuber meal (Garcia and Dale, 1999), leucaena root meal (Bhatnagar *et al*., 1996) in poultry feed. Among them cassava achieved a particular attention for its high production yield and high content of starch. Therefore, it has been identified as a possible replacement for grain in the diet of animals, if properly supplemented with protein, minerals, and in some cases, essential vitamins.

1. Ecology of cassava plant

Cassava (*Manihot esculenta*, Crantz), variously designated as manioc, yucca, or tapioca, is native to South America and Southern and Western Mexico. Cassava is a tropical crop, a perennial shrub of the *Euphorbiaceae* family, distributed between latitudes 30˚N and 30˚S. The ideal growth temperature range is 24-30˚C but it can tolerate temperatures ranging from 16 to 38˚C (Alves, 2002). It was one of the first crops to be domesticated, and there is archaeological evidence that it was grown in Peru 4,000 years ago and in Mexico 2,000 years ago (Okigbo, 1980). Htun (1990) reported that cassava was introduced into Myanmar in the middle of the 19^{th} century and was first grown in the coastal and river delta regions of the country. Cassava is a starchy staple whose roots are very rich in carbohydrates, a major source of energy. Coursey and Haynes (1970) reported that cassava can produce 250 x 10^3 calories/ha/day

compared to 176 x 10^3 for rice, 110 x 10^3 for wheat, 200 x 10^3 for maize, and 114 x 10^3 for sorghum. Although cassava roots are rich in calories, they are grossly deficient in protein, fat and some of the minerals and vitamins (Okigbo, 1980). Cassava leaves are much richer in protein than the roots, although the leaves contain a lower proportion of methionine than the root protein. The levels of all other essential amino acids in leaf protein exceed the FAO's recommended reference patterns (Eggum, 1970; FAO, 1990).

2. Cyanide toxicity in cassava

Cassava is a widely grown root crop accumulates two cyanogenic glycosides, linamarin and lotaustrain. Linamarin accounts for more than 80% of the cassava cyanogenic glycoside (White *et al*., 1998). When plant cells are damaged by wilting, frosting or stunting, the glycoside degrades to form free HCN. Thus, plants that contain the glycoside have the potential to cause HCN toxicity when consumed by animals (Stanton and Whittier, 2006). The major constraint in cassava roots as human food and animal feed is the presence of toxic cyanogenic glycosides compounds in the tissues (Bruijn, 1971). The range of total hydrocyanic acid potential (HCNp) content of different varieties of cassava is 1-1550 mg/kg (fresh material), in the root parenchyma and 900-2000 mg/kg in the root cortex (peel) (Nambisan and Sundaresan, 1985; Cardoso *et al*., 2005). Cassava leaves contain 20-1860 mg/kg of HCNp contents

(Bradbury and Denton, 2011). The concentrations of cyanogens vary in different varieties, between tissues in the same plant and even between compartments of the same tissue (Bruijn, 1971).

The sign of cyanide poisoning include excitement, staggering, paralysis, convulsions, coma and death (Burritt and Provenza, 2000). Lethal doses of HCNp in mg/kg body weight were reported 3.7 for mouse and 2.0 for cattle and sheep (Conn, 1979). It was also suggested by Gomez (1991) that maximum concentration of HCNp in animal feeds is 100 mg/kg on dry matter basis. It is therefore necessary to reduce their levels in cassava leaves and tuber to a minimum before utilization as animal feed.

3. Utilization of cassava

In South America, cassava is used mainly for animal feed (about one-third) followed by human consumption then starch production. In Asia, consumption of fresh roots and exportation to the European Union for use in animal feed are important, but its use for biofuel production is increasing (Jansson *et al*., 2009). Cassava leaves have good potential as an animal feed in the tropics on the basis of their protein, amino acid and mineral contents (Ravindran *et al*., 1982). Moreover, various parts of cassava plant, including tubers, stems and leaves are used for animal feeding. The leaves may be used as silage, dried for feed supplementation and as leaf meal for feed concentrates feeding.

The roots may be chipped and pelletized and used as feed. As the presence of HCNp constitutes a major limitation to the use of cassava in animal feed, there is the need to review current findings for the elimination of the toxic HCNp in cassava products and also to examine the implications of feeding cassava and its products on livestock production. Therefore, effective techniques for reducing the level of HCNp in both cassava leaves and tuber are necessary as making the products safe for use as feed.

4. Methods for reducing cyanide

Cassava processing improves palatability, increases shelf-life, facilitates transport and, most importantly, detoxifies cassava by removing cyanogens (Nyirenda *et al*., 2011). Upon enzymatic hydrolysis during practical processing, HCNp is liberated from linamarin and lotaustralin, rendering the dried product as a safe feed for all classes of livestock and poultry. Some methods removed nearly all residual cyanogens (Nambisan and Sundaresan, 1985) but many leave appreciable amounts behind (Cardoso *et al*., 1998). Mlingi *et al*. (1992) stated that the residual glycosides in the products have been incriminated for the toxicity associated with the continued ingestion of insufficiently processed cassava and it may still causes some degree of deleterious effects on the performance of animal. On the other hands, traditional detoxification methods do not ensure the safety of cassava with high HCNp content of cassava varieties. It is therefore

clear that attempts made to reduce the HCNp content of cassava to safe levels by using processing techniques alone are not successful. Hence, manipulation of the growing conditions such as fertilizer application and harvesting management are also very crucial to produce cassava varieties with low HCNp content, together with detoxification processes, can yield a low toxicity product. Therefore, the purpose of this study was to clarify the reduction in poisonous compounds of HCNp content and the utilization of cassava leaves and tuber as feed for monogastric animals especially, poultry. Therefore, the present study was undertaken with the following objectives:

1) To evaluate an effective processing technique to reduce HCNp content (Chapter 2 and 3);
2) To produce low cyanide cassava varieties for utilization as suitable cassava feed (Chapter 4 and 5);
3) To clarify the safe utilization of cassava leaves and tuber as feed for poultry (Chapter 6 and 7).

CHAPTER 2

Comparison of feed conditioning techniques to reduce cyanide contained in two varieties of cassava tuber

Abstract

The effects of two processing methods (soaking and wilting) and three processing periods (12 h, 24 h, 48 h) on the hydrocyanic acid potential (HCNp) of two varieties of cassava tuber were evaluated. The HCNp content was significantly higher ($P<0.05$) in the Red cassava compared with the White cassava. However, both processing methods were equally effective in lowering the HCNp content in both of Red and White cassava varieties. Moreover, results of this experiment showed that the HCNp content of soaking method at 48 h processing period was significantly lower than that of wilting in both cassava varieties. The reduction rate of HCNp contents were increased with increase of processing time from 12 h to 48 h. The soaking method produced 61.76-66.28% of HCNp reduction after 48 h in both cassava varieties. Meanwhile, the wilting method gives 50.86-51.50% of HCNp reduction in both cassava varieties. Therefore, the soaking method is efficient for reducing HCNp content in both cassava varieties within two days.

Introduction

Cassava (*Manihot esculenta*, Crantz) is a staple food in most tropical regions, and is grown over a range of climates and altitudes and on a wide variety of soils. However, cassava tuber has certain drawbacks: its tissues contain toxic cyanogenic compounds and it has a very low protein content and a very short shelf-life in fresh form of 1-3 days (Rickard, 1985). Cassava varieties are often described as being bitter or sweet by reference to the taste of fresh roots and this partly correlates with cyanogen concentrations. Bitter varieties are associated with high concentrations of cyanogenic glycosides (>100 mg/kg FM) (Chiwona-Karltun *et al*., 2004). Sweet varieties have a high concentration of free sugars but it does not always follow that they have low concentrations of cyanogenic glycoside (King and Bradbury, 1995). However, bitter taste and high level of cyanogens can also be related to environmental stress conditions, such as drought, low soil fertility and pest attack (Bruijn, 1971).

The cassava roots need to be processed if these negative aspects are to be overcome, and experienced cassava producers are aware of different ways and methods of processing cassava (Nyirenda *et al*., 2011). Processing methods used traditionally are sun-drying, soaking and fermentation followed by roasting. In the humid tropics, especially in the wet season, sun-drying in southern part of Myanmar is rather difficult

because in most cases the optimal sun-drying period coincides with the wet season which limits sun-drying of forage material. In the case of sun-drying are difficult in the rainy season, wilting under a roof and soaking promises to be a simpler and more reliable procedure. Drying by artificial means is a costly process requiring substantial investment and operational costs, and may not be a feasible idea under farm situations in developing countries. Presently, there is limited information on the comparative effects of these two methods on the HCNp content. The two processing methods, soaking and wilting, were compared and the most optimal processing period for reducing HCNp was chosen among these processing methods in two varieties of cassava tuber in this study.

Materials and Methods

Cassava tubers of two local varieties were prepared for soaking and wilting processes, respectively. From their physical appearance, one variety was named as ‘Red’, and the other was ‘White’. Those two varieties were planted in marl (pH 7.8) soil in the experimental field of the University of the Ryukyus, Okinawa, Japan, in 2010. There was no fertilizer application at the time of planting.

1. Soaking method

Approximately 100 g of unpeeled cassava roots were washed and immersed in distilled water in a clean plastic container. The temperature of all containers was maintained at room temperature at 23˚C. Each material was treated with 3 trial treatments in both varieties with three replications. Samples were collected for chemical analysis immediately at three occasions, 12, 24 and 48 h after soaking. The reduction rate of HCNp contents was determined in both cassava varieties in this study.

2. Wilting method

Fresh cassava roots were washed and weighed for approximately 100 g in both of cassava varieties. All cassava roots were spread out in the paper under the roof at 23˚C and left to wilt for 12, 24 and 48 h. Sampling was done the same procedure as described in soaking method.

3. Chemical analysis

Dry matter (DM) content of experimental material was determined by oven drying at 70˚C to a constant weight. Determinations were made of crude protein (CP), crude fiber (CF), ether extract (EE), and ash using the procedures described by AOAC (1985). Cassava tubers were analyzed for the measurement of HCNp contents using acid hydrolysis method (Bradbury *et al*., 1991; Haque and Bradbury, 2002).

4. Statistical analysis

The statistical analyses of the data, obtained at each stage of the study were performed using the SPSS 16.0 Software by ANOVA in accordance with the General Linear Model. Differences among means with $P<0.05$ were accepted as representing statistically significant difference.

Results

The chemical compositions of cassava tuber used in this experiment were shown in Table 2.1. The HCNp content was significantly higher ($P<0.05$) in the Red cassava compared with the White cassava. In this experiment, the HCNp content of cassava roots were reduced with the time after soaking and wilting methods and it was presented in Table 2.2. The HCNp contents were significantly decreased ($P<0.05$) at 48 h of processing periods in both methods. Moreover, the HCNp content in soaking processing method was significantly ($P<0.05$) lower than that of wilting at 48 h in both varieties. The HCNp contents in Red cassava variety were observed that 139.56 mg/kg, 126.69 mg/kg and 63.11 mg/kg at the 12, 24 and 48 h soaking periods, respectively. A similar reduction of HCNp contents was observed in the White variety was 88.94 mg/kg, 80.24 mg/kg and 34.80 mg/kg in each soaking periods. The percentages of

HCNp reduction in both processing methods of Red and White cassava varieties were shown in Figure 2.1a and Figure 2.1b, respectively. The soaking method corresponds to 61.76-66.28% of HCNp reduction after 48 h in Red and White cassava varieties. Meanwhile, the wilting method corresponds to 50.86-51.50% of HCNp reduction in both cassava varieties.

Table 2.1 Composition of Red and White cassava tuber before processing

Item	Red cassava	White cassava
HCNp (mg/kg DM)	165.04±6.50[a]	103.22±0.18[b]
Dry matter (%)	27.89±0.40	29.86±0.38
Crude protein (%)	3.44±0.16	3.41±0.64
Crude fiber (%)	2.57±0.74	2.54±0.69
Ether extract (%)	0.72±0.20	0.80±0.10
Ash (%)	3.81±1.07[a]	3.49±0.65[b]
Nitrogen free extract (%)	89.46±1.88	89.76±0.60

[a-b]Values (mean ± SD) with different superscript on the same row are significantly different (P<0.05).

Table 2.2 Effects of processing methods on the HCNp content of Red and White cassava tuber

		Processing time (h)	Soaking	Wilting
Red cassava	HCNp (mg/kg DM)	0	165.04 ± 6.50^{A}	165.04 ± 6.50^{A}
		12	139.56 ± 6.38^{B}	141.68 ± 4.99^{B}
		24	126.69 ± 5.52^{Ca}	101.35 ± 7.72^{Cb}
		48	63.11 ± 6.04^{Db}	80.04 ± 6.58^{Da}
	Reduction (%)	0	-	-
		12	15.43 ± 3.86^{C}	14.14 ± 3.03^{C}
		24	23.23 ± 3.35^{Bb}	38.58 ± 4.68^{Ba}
		48	61.76 ± 3.66^{Aa}	51.50 ± 3.99^{Ab}
White cassava	HCNp (mg/kg DM)	0	103.22 ± 0.18^{A}	103.22 ± 0.18^{A}
		12	88.94 ± 1.88^{B}	88.77 ± 5.33^{B}
		24	80.24 ± 7.78^{C}	66.81 ± 8.11^{C}
		48	34.80 ± 2.40^{Db}	50.72 ± 4.70^{Da}
	Reduction (%)	0	-	-
		12	13.83 ± 3.86^{B}	14.00 ± 3.03^{C}
		24	22.25 ± 3.35^{Bb}	35.27 ± 4.68^{Ba}
		48	66.28 ± 3.66^{Aa}	50.86 ± 3.99^{Ab}

$^{A-D}$Values (mean ± SD) with different superscript on the same column are significantly different ($P<0.05$). $^{a-b}$Values (mean ± SD) with different superscript on the same row are significantly different ($P<0.05$).

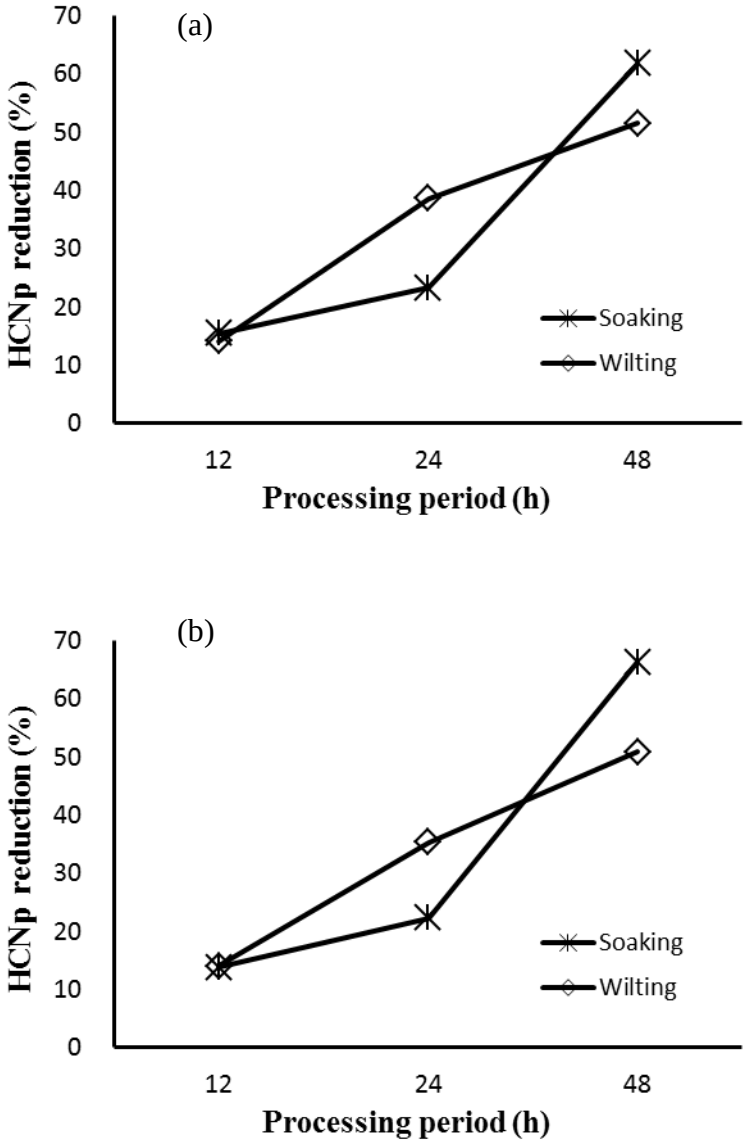


Figure 2.1 The reduction rate of HCNp content as a function of soaking and wilting time of Red cassava tuber (a) and White cassava tuber (b).

Discussion

The initial HCNp contents of both Red and White cassava varieties were different in this experiment. The HCNp content in both varieties used in this experiment was range from 103.22 to 165.04 mg/kg and these values are large differences between the HCNp content in cassava tuber reported by different researchers. According to Kalenga Saka and Nyirenda (2012), the HCNp content in cassava tuber was 1314.50 mg/kg in the peel and 306.50 mg/kg in parenchyma. These variations could be explained by the fact that the content of HCNp in cassava tuber depends on variety (Vetter, 2000). The results of this study show that increasing soaking time could reduce HCNp content in cassava roots of both varieties. O'Brien *et al*. (1992) reported that the characteristic of HCN is water soluble and volatile. Furthermore, Nambisan and Sundaresan (1985) stated that cyanoglucoside in the tuber is leached out into the water. The volume of water should be adequate for optimum dissolution of cyanoglucoside. The highest loss of HCNp contents was observed at 48 h soaking period in both cassava varieties. Oyewole and Afolami (2001) described that acid production during cassava fermentation has been attributed to the activities of the lactic acid bacteria on the carbohydrate content of cassava root. Root softening during soaking has been described to an increase in the activity of cell-wall degrading

enzymes (Ampe *et al*., 1995; Okolie and Ugochukwu, 1998). However, Adebayo-Oyetoro *et al*. (2012) reported that the more the cassava stays in the water during fermentation, the more the minerals are leached into the water thereby making them to have reduced values of protein.

The present study found that wilting also had a pronounced effect on reducing the HCNp content in both cassava varieties. This was confirmed with the statement of Hang and Preston (2005) and Phengvichith and Ledin (2007) showed that wilting fresh cassava foliage in a shed could reduce the HCNp content by 58% and 45%, respectively. In this study, the HCNp contents were reduced by 50.86% and 51.50% in both cassava varieties, when wilting in a shed for 48 h. It may be assumed that wilting provided adequate time for the linamarase to act on the cyanoglucosides of cassava root, thereby removing an appreciable amount of HCN from it (Nambisan and Sundaresan, 1985). Although Red cassava variety had higher HCNp initially, there was slightly uniform reduction in HCNp contents in the two cassava variety during soaking and wilting processes. Ravindran (1992) reported that the destruction of cell wall structures by different methods favors the intracellular reaction of linamarase with the cyanogenic glucosides present in cassava, thus contributing to a rapid HCN elimination from the material.

Soaking cassava roots for up to 48 h reduced HCNp contents to minimal values and the percentage of reduction being more pronounced than wilting process in both cassava varieties. With regard to the reduction in HCNp content during the soaking process, Cooke (1979) observed that soaking in water cause tissue cellular disruption that results in comparatively greater susceptibility to the actions of bacteria, as indicated by the fall in pH values, and the enzymes α-amalyse and endogenous linamarase. Ogbo (2006) described that the fermentation processes took place in an acid environment (6.9-3.4). Therefore, further study should be conducted to clarify the fermentation process of cassava because the population and composition of the microorganisms as well as the reduction of cyanogenic glycosides at various stages need to be evaluated. This investigation highlighted the importance of soaking cassava roots at least 48 h prior to utilization as feed for animals.

Conclusion

The results of this study showed that increasing soaking and wilting time could reduce HCNp content, respectively. The mean reductions of HCNp in both varieties were 64% (48 h) and 51% (48 h) for soaking and wilting processes, respectively. Root soaking for unpeeled tuber showed more pronounced effect on HCNp reduction.

CHAPTER 3

Effect of ensiling process and additive effects of fermented juice of epiphytic lactic acid bacteria on the cyanide content of two varieties of cassava

Abstract

Two experiments were conducted to evaluate the effects of processing method (silage making) on the hydrocyanic acid potential (HCNp) of two local varieties cassava leaf (Experiment 1) and tuber (Experiment 2). The study also ascertained the effects of fermented juice (leucaena leaves and napiergrass) of epiphytic lactic acid bacteria (FJLB) as additives and their impact on the changes of HCNp content at different duration (0, 2, 7 and 14 d) of ensiling. The reduction rate of the HCNp of cassava leaves and tuber ensiled with FJLB additives were significantly higher ($P<0.05$) than the control silage in both varieties. The reduction of HCNp in final products of FJLB treated silages ranged from 18.54 to 82.30 mg/kg DM. The lowest HCNp (Red cassava, $P = 0.007$ and White cassava, $P = 0.0001$) and the highest lactic acid bacteria counts (Red cassava, $P = 0.004$ and White cassava, $P = 0.002$) were observed after 14 d ensiling period in both varieties treated with leucaena FJLB in the second experiment. As indicated by the low HCNp level and high V-score value on 14

d after ensiling period, it is concluded that, the addition of FJLB to cassava silages was effective in optimum reduction of HCNp besides producing a good quality silage within two weeks. Moreover, a negative correlation between HCNp reduction and pH in of ensiled cassava was observed.

Introduction

Cassava farming populations have empirically developed several processing methods for stabilizing cassava and reducing its toxicity (Balagopalan *et al*., 1988). Physiological deterioration occurs in cassava roots, 2-5 days after harvesting followed by microbial deterioration 3-5 days later (Ampe *et al*., 1994). Fermentation is a process which transforms its low technology and energy requirement to a final product (Daeschel *et al*., 1987). As demonstrated in the available literatures, ensiling could be a suitable way for preserving cassava which leads to be efficient in utilization (Limon, 1991). One of the main problems encountered during ensiling of cassava is the release of large quantities of silage effluent which leads to loss of essential nutrients and also results in poor quality watery silage with very low shelf-life (Balagopalan, 2002).

Sugarcane molasses, a common feed ingredient in the tropics, already been reported as a potential additive in cassava leaves silage (Man and Wiktorsson, 2001).

The fermented juice of epiphytic lactic acid bacteria (FJLB) was recommended as a silage additive for improving the fermentative quality of tropical grass silage (Bureenok *et al*., 2005 a,b). However, it appears that no information is available on the utilization of the FJLB additive in silage making process of cassava. Tamada *et al*. (1999) also reported that the addition of this juice in napiergrass silage can improve silage quality, similar to the use of commercial lactic acid bacteria (LAB) inoculant. The species and the number of LAB vary considerably with plants, fields and season (Cai *et al*., 1998). To perform lactic fermentation more surely, tropical forage plant species such as leucaena (*Leucaena leucocephala*) and napiergrass (*Pennisetum purpureum*), which the both species are growing naturally in tropical and subtropical region, were used to make FJLB for this experiment. The influence of storage period on the hydrocyanic acid potential (HCNp) content of cassava leaf and tuber silages was of an additional interest. Therefore, the present study aimed at investigating the effects of ensiling process on reduction of the HCNp content on the two local cassava varieties. Two separate experiments were conducted using leaves (Experiment 1) and tuberous roots (Experiment 2) of cassava.

Materials and Methods

1. Plant materials

Leaves and tubers of two local varieties (Red and White) of cassava were prepared for experiment 1 and experiment 2, respectively. Cassava was collected from the field of the University of the Ryukyus, Okinawa.

Experiment 1. FJLB prepared by leucaena and napiergrass were used as an additive in silage making, whose were prepared by incubating leucaena or napiergrass juice for 2 days prior to silage making. Each 167 g of leucaena and napiergrass were macerated with 500 ml of distilled water using a blender. The macerate was then filtered through a sterilized double layer of cheesecloth and the filtrate was put into a 500 ml flask. Approximately 1% (w/v) of glucose was added into the filtrate, and was then shaken well and kept in an incubator at 30˚C for 2 days (Bureenok *et al*., 2005 a).

Cassava leaves of Red and White varieties were collected from the field immediately prior to harvesting the tubers. The leaves with petioles were separated from the stem and chopped into small pieces (1-2 cm). The chopped pieces were mixed with leucaena-FJLB and napiergrass-FJLB (both at 1%, w/v) while filling and the materials were sealed with a vacuum packaging machine (Vacuum sealer, SQ 202, Sharp Co. Ltd., Osaka, Japan). The control silage was added with an equivalent

amount of distilled water 1% (v/w). Approximately 100 g of chopped cassava with three replications for each treatment were ensiled in each bag. The silos were kept at 23˚C and samples were taken on the 0, 2, 7 and 14 d after ensiling for chemical analysis.

Experiment 2. The FJLB was prepared as described in experiment 1. Cassava tuber of Red and White varieties were harvested in May, 2011. They were ensiled with leucaena FJLB 1% (w/v) or napiergrass FJLB 1% (w/v), respectively. Three replicate pouches were prepared for each treatment and all pouches were stored at 23˚C and samples were taken on the 0, 2, 7 and 14 d after ensiling for chemical analysis.

2. Chemical analysis

Dry matter (DM) content of silage was determined by oven drying at 70˚C to a constant weight. Determinations were made of CP, CF, EE, and ash using the procedures described by AOAC (1985). The HCNp content was analyzed by using acid hydrolysis method described by Bradbury *et al*. (1991) and Haque and Bradbury (2002). The pH values of cassava silages were measured by using a pH meter (F-23; Horiba Co. Ltd., Tokyo, Japan). The changes in the number of LAB during fermentation were examined by using GYP-$CaCO_3$ agar at 35˚C for 3 d. Colonies were counted as viable numbers of microorganisms, and are expressed as colony-forming

unit per ml (log_{10} cfu/ml) (Kozaki *et al.*, 1992). Ammonia-nitrogen (NH_3-N) was analyzed by using a steam distillation technique (JGFFSA, 1994). The volatile fatty acids (VFA) content was determined by using HPLC (Shim-pack SCR-102H, 300 mm x 8.0 mm i.d; column temperature 40˚C; flow rate 0.8 ml/min, Shimadzu Co. Ltd., Kyoto, Japan). V-score, which was used to assess the silage fermentation quality, was calculated on the values of organic acids and NH_3-N content as per JGFFSA (1994).

3. Statistical analysis

The statistical analyses of the data were performed using the General Linear Model procedure of SPSS 16.0 Software. The means were separated using Duncan's Multiple Range Test by the same software.

Results

Experiment 1. The chemical composition of the cassava leaves before silage making are shown in Table 3.1. The HCNp value was significantly ($P<0.05$) higher in the Red cassava compared with the White cassava. The reduction in HCNp values were similar to control on 2 d post-ensiling, but decreased significantly ($P<0.05$) in both leucaena- and napier-FJLBs as the ensiling period increased, especially after 7 d of ensiling, irrespective of the varieties (Table 3.2). In spite of the different cassava varieties used,

similar mean reduction levels (74.67 and 72.33% for leucaena-FJLB and 79.00 and 77.67% for napier-FJLB) in HCNp values were observed on 14 d after ensiling with FJLBs. In both cases, the corresponding reduction in HCNp in control silage was much lower ($P<0.05$). According to the results of HCNp reduction level, no differences ($P<0.05$) was apparent between the source of FJLB i.e. leucaena- and napier-FJLBs at 14 d ensiling period for both cassava varieties.

The fermentation characteristics of the Red and White cassava silages using cassava leaves are presented in Tables 3.3 and 3.4, respectively. The overall pH values of Red and White cassava leaf silages tended to decrease from 2 d to 14 d after ensiling. A significant ($P<0.05$) effect of FJLBs addition on pH value was found on the 7 d and 14 d after ensiling in both cassava varieties, when compared to the respective control values. Further, a pattern of a gradual decrease of pH was observed in both Red and White cassava leaves silages accompanying the increasing rate of HCNp reduction (Figure 3.1a and 3.1b).

The number of LAB (log_{10} cfu/ml) was significantly higher ($P<0.05$) in both the FJLB treatments than the control in both cassava varieties at 14 d post-ensiling. Moreover, napier-FJLB had significantly ($P<0.05$) higher LAB counts than leucaena-FJLB in case of White cassava (Table 3.4). The butyric acid contents at 14 d in the

control silage were 9.36 and 6.70%, respectively, of Red and White cassava varieties; but in case of the FJLB treated silages, butyric acid was not detected.

Experiment 2. The chemical composition of cassava tubers before silage making is presented in Table 3.5. The HCNp value was significantly ($P<0.05$) higher in the Red cassava compared with the White cassava. The HCNp values decreased significantly ($P<0.05$) with the progression of ensiling period in both the varieties (Table 3.6). The rate of reduction in HCNp because of ensiling of cassava tuber of both varieties was somewhat similar. On 14 d post-ensiling, the HCNp content in FJLB-treated silages were significantly lower ($P<0.05$) than the control in both varieties. The percent reduction in HCNp in the FJLB-treated silages ranged from 76.67 to 84.33% across the varieties. In case of Red cassava tuber, the percent reduction was higher ($P<0.05$) with leucaena-FJLB than the control or napier-FJLB. However in case of White variety, the reduction was higher in both leucaena- and napier-FJLB treatments as compared to the control.

The fermentative quality data of Red and White cassava tuber silages are shown in Tables 7 and 8, respectively. The decline in pH was gradual and reached the minimum values at 14 d post-ensiling, irrespective of variety and additive use. However, the drop in pH was significantly ($P<0.05$) higher in both the FJLB-treated

silages than their respective control values, irrespective of the cassava varieties. Similar to the results in Experiment 1, the pH level declination was correlated with the increasing rate of HCNp reduction (Figure 3.2a and 3.2b).

The LAB counts in FJLB-treated silages were significantly ($P<0.05$) higher than the control on 14 d post-ensiling of cassava tuber in both the varieties; however, the leucaena-FJLB treated silages showed higher ($P<0.05$) LAB counts than the napier-FJLB in both the varieties. Butyric and propionic acids were not detected in any of the silages. The content of NH_3-N was significantly lower ($P<0.05$) on the 14 d than 2 d and 7 d post-ensiling in both cassava varieties. Further, there was no effects of addition of the two FJLBs on the NH_3-N levels. A significantly ($P<0.05$) higher V-score value with FJLB treatment was evident than the control in White cassava with no such variations apparent in case of the Red variety.

Table 3.1 Chemical composition of Red and White cassava leaves before silage making

Item	Red cassava	White cassava
HCNp (mg/kg DM)	325.25±9.02[a]	268.46±6.60[b]
Dry matter (%)	26.48±0.40[b]	27.78±0.38[a]
Crude protein (% DM)	10.46±0.16[b]	12.28±0.64[a]
Crude fiber (% DM)	15.60±0.74[a]	13.75±0.69[b]
Ether extract (% DM)	4.50±0.20[a]	4.10±0.10[b]
Ash (% DM)	9.47±1.07[b]	11.47±0.65[a]
Nitrogen free extract (% DM)	59.61±1.88	58.39±0.60

[a-b]Values (mean ± SD) with different superscript on the same row are significantly different ($P<0.05$).

Table 3.2 Effects of addition of FJLB prepared with leucaena or napiergrass on the HCNp content of Red and White cassava leaves silage

	Days post-ensiling	Silage additive		
		Control	Leucaena-FJLB	Napier-FJLB
Red cassava				
HCNp (mg/kg DM)	2	224.45 ± 5.54^{A}	219.11 ± 4.49^{A}	206.55 ± 6.03^{A}
	7	185.77 ± 8.71^{Ba}	159.79 ± 5.39^{Bb}	152.68 ± 5.39^{Bb}
	14	154.97 ± 8.24^{Ca}	82.30 ± 5.74^{Cb}	68.90 ± 6.19^{C}
Reduction (%)	2	31.00 ± 1.70^{C}	32.67 ± 1.38^{C}	36.33 ± 2.30^{C}
	7	42.67 ± 3.05^{Bb}	50.67 ± 1.66^{Ba}	52.67 ± 1.66^{Ba}
	14	52.67 ± 2.51^{Ac}	74.67 ± 1.52^{Ab}	79.00 ± 2.00^{A}
White cassava				
HCNp (mg/kg DM)	2	193.82 ± 6.27^{A}	189.63 ± 8.69^{A}	189.62 ± 2.61^{A}
	7	165.70 ± 2.07^{Ba}	152.54 ± 7.16^{Bab}	140.27 ± 2.75^{Bb}
	14	135.62 ± 8.57^{Ca}	73.96 ± 7.35^{Cb}	59.58 ± 2.53^{Cc}
Reduction (%)	2	27.67 ± 2.08^{C}	29.33 ± 3.05^{C}	29.00 ± 1.00^{C}
	7	38.33 ± 1.15^{Bb}	43.33 ± 2.88^{Bab}	47.67 ± 1.02^{Ba}
	14	49.33 ± 3.21^{Ac}	72.33 ± 2.51^{Ab}	77.67 ± 1.15^{A}

$^{A-C}$Values (mean ± SD) with different superscript on the same column are significantly different ($P<0.05$). $^{a-c}$Values (mean ± SD) with different superscript on the same row are significantly different ($P<0.05$).

Table 3.3 Effects of addition of FJLB prepared with leucaena or napiergrass on the quality of Red cassava leaves silage

Item	Days post-ensiling	Silage additive		
		Control	Leucaena-FJLB	Napier-FJLB
pH	2	6.17 ± 0.07^{A}	5.58 ± 0.27^{A}	5.58 ± 0.51^{A}
	7	5.67 ± 0.01^{Ba}	4.82 ± 0.25^{Bb}	4.95 ± 0.24^{Ab}
	14	5.38 ± 0.17^{Ca}	4.14 ± 0.14^{Cb}	4.56 ± 0.13^{Bb}
Lactic acid bacteria	2	3.53±0.68	4.93 ± 0.29^{C}	4.15 ± 1.00^{B}
(log_{10} cfu/ml)	7	4.45 ± 0.39^{b}	6.43 ± 0.58^{Ba}	6.04 ± 1.02^{Aab}
	14	4.42 ± 0.10^{b}	7.42 ± 0.13^{Aa}	6.94 ± 0.83^{Aa}
Lactic acid	2	1.02 ± 0.70^{b}	1.59 ± 0.07^{Ab}	2.80 ± 0.46^{Aa}
(% DM)	7	1.08 ± 0.06^{c}	1.48 ± 0.05^{Ab}	1.74 ± 0.09^{Ba}
	14	1.00±0.29	1.22 ± 0.02^{B}	1.57 ± 0.48^{B}
Acetic acid	2	2.24 ± 1.48^{B}	0.99 ± 0.71^{B}	2.17 ± 0.27^{C}
(% DM)	7	2.91 ± 0.49^{B}	2.17 ± 1.70^{B}	2.96 ± 0.28^{B}
	14	4.94 ± 0.70^{A}	4.88 ± 0.74^{A}	3.52 ± 0.06^{A}
Propionic acid	2	ND	ND	ND
(% DM)	7	0.81 ± 0.18^{B}	ND	ND
	14	17.89 ± 1.29^{Aa}	0.63 ± 0.01^{b}	0.62 ± 0.02^{b}
Butyric acid	2	ND	ND	ND
(% DM)	7	ND	ND	ND
	14	9.36±0.61	ND	ND
NH_3-N	2	14.90 ± 1.18^{A}	13.60±3.80	12.51±3.61
(% TN)	7	14.33 ± 1.06^{Aa}	12.68 ± 1.25^{ab}	11.00 ± 1.65^{b}
	14	11.34 ± 1.10^{Ba}	9.69 ± 0.55^{ab}	8.72 ± 1.41^{b}
V-score	2	96.92 ± 3.04^{A}	99.13 ± 1.47^{A}	96.93 ± 0.62^{A}
	7	94.02 ± 0.66^{A}	97.09 ± 3.40^{A}	95.56 ± 0.49^{B}
	14	50.00 ± 23.09^{Bb}	90.67 ± 0.96^{Ba}	93.04 ± 0.16^{Ca}

Ammonia-nitrogen (% total nitrogen). $^{A-C}$Values (mean ± SD) with different superscript on the same column are significantly different ($P<0.05$). $^{a-c}$Values (mean ± SD) with different superscript on the same row are significantly different ($P<0.05$).
ND: Not detected

Table 3.4 Effects of addition of FJLB prepared with leucaena or napiergrass on the quality of White cassava leaves silage

Item	Days post-ensiling	Silage additive		
		Control	Leucaena-FJLB	Napier-FJLB
pH	2	5.60 ± 0.07^{Aa}	4.54 ± 0.11^{Ab}	5.06 ± 0.45^{Aab}
	7	5.17 ± 0.09^{Ba}	4.38 ± 0.31^{Ab}	4.33 ± 0.27^{Bb}
	14	4.84 ± 0.15^{Ca}	3.77 ± 0.08^{Bb}	3.80 ± 0.04^{Bb}
Lactic acid bacteria	2	3.97 ± 0.85	4.23 ± 0.58^{B}	4.60 ± 0.17^{B}
(log_{10} cfu/ml)	7	4.01 ± 0.41^{b}	5.88 ± 0.25^{Aa}	5.26 ± 1.10^{Bab}
	14	3.67 ± 0.57^{c}	6.23 ± 0.40^{Ab}	7.60 ± 0.43^{Aa}
Lactic acid	2	1.15 ± 0.37	1.35 ± 0.13^{A}	1.63 ± 0.38
(% DM)	7	1.04 ± 0.24^{b}	1.50 ± 0.11^{Aa}	1.70 ± 0.25^{a}
	14	1.07 ± 0.04^{b}	1.07 ± 0.05^{Bb}	1.29 ± 0.06^{a}
Acetic acid	2	4.18 ± 0.43^{a}	2.64 ± 0.31^{b}	1.21 ± 0.01^{Bc}
(% DM)	7	4.52 ± 0.45^{a}	4.06 ± 1.12^{a}	2.28 ± 0.12^{Ab}
	14	4.57 ± 0.05^{a}	4.00 ± 0.34^{a}	2.83 ± 0.50^{Ab}
Propionic acid	2	1.42 ± 0.98^{B}	ND	ND
(% DM)	7	2.38 ± 0.17^{Ba}	0.68 ± 0.20^{Bb}	ND
	14	3.79 ± 0.68^{Aa}	1.55 ± 0.14^{Ab}	0.38 ± 0.01^{c}
Butyric acid	2	ND	ND	ND
(% DM)	7	4.90 ± 2.71	ND	ND
	14	6.70 ± 0.11	ND	ND
NH_3-N	2	14.79 ± 2.08^{A}	13.96 ± 1.27^{A}	12.07 ± 1.76^{A}
(% TN)	7	13.32 ± 0.42^{Aa}	11.86 ± 1.03^{Ab}	12.51 ± 0.47^{Aab}
	14	11.73 ± 0.83^{Ba}	9.06 ± 0.82^{Bb}	8.50 ± 0.40^{Bb}
V-score	2	90.48 ± 0.83^{Ac}	96.02 ± 0.67^{Ab}	98.97 ± 0.03^{Aa}
	7	50.00 ± 0.00^{Bc}	92.21 ± 2.02^{Bb}	96.84 ± 0.17^{Ba}
	14	50.00 ± 0.00^{Bc}	90.28 ± 0.49^{Bb}	94.86 ± 1.01^{Ca}

Ammonia-nitrogen (% total nitrogen). $^{A-C}$Values (mean ± SD) with different superscript on the same column are significantly different ($P<0.05$). $^{a-c}$Values (mean ± SD) with different superscript on the same row are significantly different ($P<0.05$).
ND: Not detected

Table 3.5 Chemical composition of Red and White cassava tuber before silage making

Item	Red cassava	White cassava
HCNp (mg/kg DM)	185.14±20.4[a]	116.94±12.11[b]
Dry matter (%)	28.87±0.91	30.50±2.23
Crude protein (% DM)	3.42±0.04[a]	3.12±0.01[b]
Crude fiber (% DM)	2.56±0.21	2.41±0.22
Ether extract (% DM)	0.73±0.01	0.81±0.04
Ash (% DM)	3.80±0.10[a]	3.48±0.09[b]
Nitrogen free extract (% DM)	89.49±0.19[b]	90.18±0.30[a]

[a-b]Values (mean ± SD) with different superscript on the same row are significantly different ($P<0.05$).

Table 3.6 Effects of addition of FJLB prepared with leucaena and napiergrass on the HCNp content of Red and White cassava tuber silage

	Days post-ensiling	Silage additive		
		Control	Leucaena-FJLB	Napier-FJLB
Red cassava				
HCNp (mg/kg DM)	2	124.21 ± 3.77^{A}	117.23 ± 6.09^{A}	122.94 ± 2.92^{A}
	7	83.17 ± 2.90^{B}	75.89 ± 5.52^{B}	82.00 ± 4.53^{B}
	14	48.44 ± 8.53^{Ca}	25.13 ± 2.26^{C}	32.43 ± 4.94^{Cb}
Reduction (%)	2	30.67 ± 3.51^{C}	32.00 ± 2.00^{C}	31.33 ± 3.51^{C}
	7	52.33 ± 1.57^{B}	55.33 ± 3.78^{B}	53.33 ± 2.44^{B}
	14	70.33 ± 7.76^{Ab}	84.33 ± 2.30^{A}	80.00 ± 2.64^{Aab}
White cassava				
HCNp (mg/kg DM)	2	78.89 ± 6.31^{A}	77.37 ± 3.60^{A}	78.56 ± 6.89^{A}
	7	53.73 ± 4.40^{Ba}	46.13 ± 2.81^{B}	48.50 ± 2.42^{Bab}
	14	30.87 ± 0.80^{Ca}	18.54 ± 1.49^{Cc}	24.86 ± 1.61^{Cb}
Reduction (%)	2	31.68 ± 3.05^{C}	33.00 ± 2.64^{C}	32.33 ± 2.08^{C}
	7	52.33 ± 1.52^{Bb}	58.33 ± 1.15^{Ba}	55.67 ± 3.05^{Bab}
	14	71.67 ± 0.58^{Ac}	83.00 ± 2.00^{A}	76.67 ± 0.59^{Ab}

$^{A-C}$Values (mean ± SD) with different superscript on the same column are significantly different ($P<0.05$). $^{a-c}$Values (mean ± SD) with different superscript on the same row are significantly different ($P<0.05$).

Table 3.7 Effects of addition of FJLB prepared with leucaena and napiergrass on the quality† of Red cassava tuber silage

Item	Days post-ensiling	Silage additive		
		Control	Leucaena-FJLB	Napier-FJLB
pH	2	4.60 ± 0.00^{Aa}	4.56 ± 0.04^{Aa}	4.37 ± 0.04^{Ab}
	7	4.11 ± 0.01^{Bb}	4.18 ± 0.02^{Ba}	4.09 ± 0.01^{Bb}
	14	4.00 ± 0.02^{Ca}	3.87 ± 0.05^{Cb}	3.90 ± 0.08^{Cab}
Lactic acid bacteria	2	3.10 ± 0.17^{Bb}	4.87 ± 0.75^{Ca}	5.21 ± 0.49^{Ba}
(log_{10} cfu/ml)	7	4.46 ± 0.36^{ABb}	5.91 ± 0.14^{Ba}	5.36 ± 0.33^{Aab}
	14	5.55 ± 0.41^{Ac}	7.98 ± 0.77^{Aa}	6.30 ± 0.30^{Ab}
Lactic acid	2	2.96 ± 0.11^{B}	3.73 ± 0.77^{B}	3.43 ± 0.19^{C}
(% DM)	7	4.62 ± 0.29^{A}	4.64 ± 0.86^{AB}	4.86 ± 0.22^{A}
	14	4.94 ± 0.72^{Aab}	5.52 ± 0.50^{Aa}	4.34 ± 0.18^{Bb}
Acetic acid	2	1.07 ± 0.13^{B}	0.97 ± 0.09	1.14 ± 0.04^{AB}
(% DM)	7	1.28 ± 0.04^{Aa}	1.05 ± 0.16^{b}	1.27 ± 0.07^{Aa}
	14	1.23 ± 0.07^{AB}	1.17 ± 0.05	1.08 ± 0.02^{B}
NH_3-N	2	13.53 ± 1.71^{A}	13.69 ± 0.98^{A}	13.88 ± 0.99^{A}
(% TN)	7	13.01 ± 0.25^{A}	12.80 ± 1.16^{A}	13.00 ± 1.56^{A}
	14	7.80 ± 0.85^{B}	7.20 ± 0.97^{B}	6.97 ± 0.21^{B}
V-score	2	98.96±0.25	98.90±0.23	98.79±0.14
	7	98.60±0.11	98.60±0.10	98.88±0.40
	14	98.35±0.13	99.00±0.20	98.40±0.06

Ammonia-nitrogen (% total nitrogen). $^{A-C}$Values (mean ± SD) with different superscript on the same column are significantly different ($P<0.05$). $^{a-c}$Values (mean ± SD) with different superscript on the same row are significantly different ($P<0.05$).

†Concentrations of propionic acid and butyric acid were non-detectable

Table 3.8 Effects of addition of FJLB prepared with leucaena and napiergrass on the quality† of White cassava tuber silage

Item	Days post-ensiling	Silage additive		
		Control	Leucaena-FJLB	Napier-FJLB
pH	2	4.53±0.05^{A}	4.55±0.08^{A}	4.47±0.03^{A}
	7	4.16±0.01^{Ba}	4.03±0.03^{Bb}	4.00±0.01^{Bb}
	14	4.02±0.03^{Ca}	3.84±0.06^{Cb}	3.89±0.05^{Cb}
Lactic acid bacteria	2	3.16±0.28^{Cb}	4.77±0.68^{Ca}	4.43±0.51^{Cab}
(log_{10} cfu/ml)	7	4.17±0.08^{Bb}	5.83±1.19^{Ba}	5.61±0.41^{Bab}
	14	5.38±0.29^{Ac}	7.85±0.52^{Aa}	6.62±0.53^{Ab}
Lactic acid	2	3.47±0.47^{B}	4.67±1.10	3.70±0.95^{B}
(% DM)	7	4.93±1.25^{AB}	5.50±0.17	5.03±0.15^{A}
	14	5.40±0.17^{A}	5.83±0.50	5.37±0.46^{A}
Acetic acid	2	1.23±0.17	1.01±0.11	1.02±0.17
(% DM)	7	1.30±0.45	1.05±0.07	1.23±0.18
	14	1.30±0.02^{a}	1.11±0.06^{b}	1.04±0.09^{b}
NH_3-N	2	16.32±1.60^{A}	14.58±0.81^{A}	14.54±2.57^{A}
(% TN)	7	15.18±2.36^{A}	13.85±1.97^{A}	13.75±1.07^{A}
	14	8.87±0.54^{B}	8.17±0.39^{B}	8.05±1.83^{B}
V-score	2	98.76±0.56	98.90±0.28	98.40±0.32
	7	98.61±0.80	98.60±0.34	99.09±0.05
	14	98.41±0.06^{b}	99.00±0.03^{a}	98.88±0.09^{a}

Ammonia-nitrogen (% total nitrogen). $^{A-C}$Values (mean ± SD) with different superscript on the same column are significantly different ($P<0.05$). $^{a-c}$Values (mean ± SD) with different superscript on the same row are significantly different ($P<0.05$).

†Concentrations of propionic acid and butyric acid were non-detectable

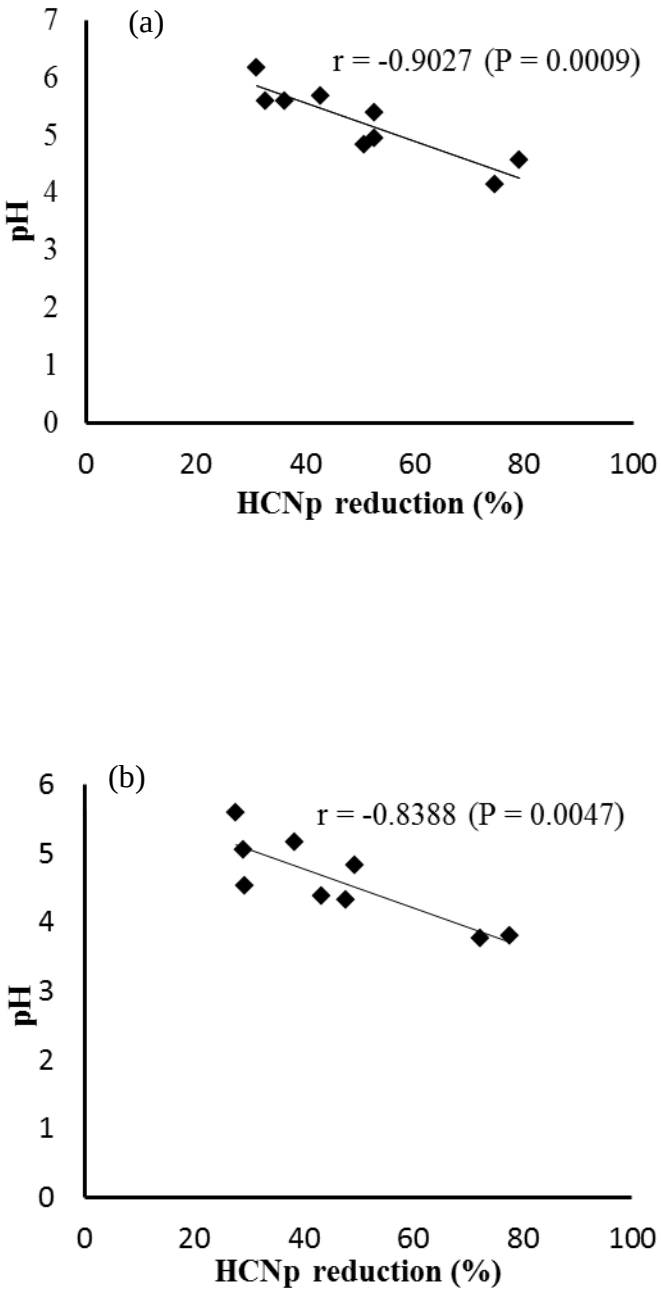


Figure 3.1. Reduction rate of HCNp content with the declination of pH value in Red cassava leaves (a) and White cassava leaves (b) silage.

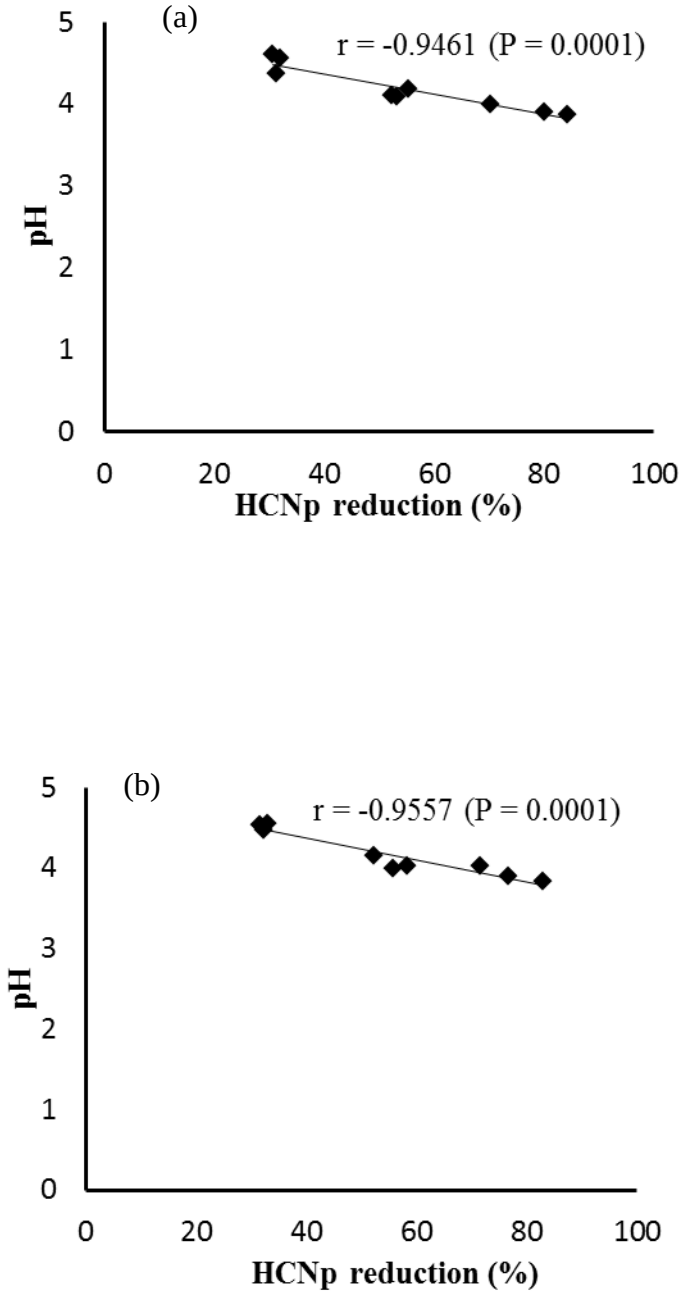


Figure 2.2. Reduction rate of HCNp content with the declination of pH value in Red cassava tuber (a) and White cassava tuber (b) silage.

Discussion

Although cassava leaves are rich in protein compared with cassava tuber, factors such as high crude fiber may limit its nutritive value for monogastric animals (Ravindran, 1991). The present results concerning CP content of cassava tuber are in agreement with those of Emmanuel *et al*. (2012) whose investigation of six cassava varieties reported CP values ranging from 1.2 to 3.5%. In the present study, there was quite a variation in HCNp values between Red and White cassava varieties in both the experiments. In literature, the ranges of HCNp content of different varieties of cassava varies from 1 to 1550 mg/kg (Cardoso *et al*., 2005). The variation observed in HCNp content of cassava has been attributed to genetic, physiological, edaphic and climatic differences (Gomez and Valdivieso, 1985). In the present study, the environmental conditions such as the soil, fertilization and climate were equal, thus it is probable that the major factor causing variations in HCNp contents was of genetic-origin.

Over 70% elimination of the HCNp was observed in the final products of FJLB-treated silages in both the experiments. The fermentation must be thus a detoxification process which probably was caused due to the cyanide-degrading activities of fermenting microorganisms. During the ensiling process, chopping during the preparation before sampling, pressing and the initial environment of the aerobic

phase resulted in good conditions for reducing the HCNp content. The additive effect of napier-FJLB on the HCNp reduction were more pronounced in cassava leaves while leucaena-FJLB was more effective in reducing HCNp in cassava tubers. It is thus possible that the additive effects of FJLB depend on the nutrient composition of the parent material used for silage making such as leaves and tubers of cassava in this experiment. Therefore, it could be inferred that the additive effect of FJLB will very depending not only on the kind of forage used for FJLB treatment but also on the parts of cassava used in silage making. Woolford (1984) also reported that the bacterial flora of living plant materials is different from that of harvested plants and fermented products.

Similar to our observation, a reduction of pH from 7.1 to 3.5-4.0 after 7-21 days of ensiling was observed by Hang (1998), in which sugarcane molasses or rice bran was used as an additive for ensiling cassava leaves. The decrease in pH during fermentation of cassava tuber resulted from the production of lactic acids by LAB, which constituted the dominant microflora (Kobawila, 2005). Therefore, Lin *et al*. (1992) also reported that the LAB should be applied at a higher rate than the existing epiphytic bacterial population on the crop, which will encourage early lactic acid production and increase the rate of pH decline. Moreover, Vasconcelos *et al*. (1990)

reported that hydrolysis of cyanogenic glucosides takes place in acid environment (pH 3.8) during lactic fermentation as well as in basic environment (pH 8.5) during alkaline fermentation of the cassava.

Considering the pattern of LAB count, it was clearly evident that it increased with the extension of the ensiling period. This confirmed the report of Brauman *et al*. (1996) which explained that the fermentation of LAB in cassava began on the first 2 d of fermentation, and then increased rapidly and remained stable until the 14 d. According to the results of present experiments (1 and 2), the low HCNp contents were observed together with high LAB counts on 14 d post-ensiling with FJLBs as additive, in both varieties of cassava. It is well established that LAB play an important role in silage fermentation. Epiphytic micro-flora present on forage crops is responsible for silage fermentation and influenced silage quality (Lin *et al*., 1992; Cai *et al*., 1998). Kobawila *et al*. (2005) also reported that many strains of LAB possess linamarase activity and eliminate cyanogenic glucosides by the action of the bacterial enzymes. The linamarase produced by the cassava LAB, notably *Leuconostoc mesenteroides* and *Lactococcus lactis*, and the endogenous linamarase contribute to the process of detoxification. Thus increase amount of the enzyme will quicken the process of degradation of cyanogenic glucoside and liberation of cyanide (Ahaotu *et al*., 2013).

Although FJLBs contain many kinds of LAB, further research may be needed to identify the actual effective species of LAB, and to clarify the mechanism of bacteria for reducing the HCNp content in cassava.

In Experiment 1, the pH value of the control silage did not decline to <4.5 and it led to the butyric fermentation (Cai *et al*., 1998). On the other hand, butyric acid content of the silage without FJLB additives was 6-9% in cassava leaves silage. McDonald (1991) stated that silages containing more than 10% butyric acid are poorly preserved. The higher percentage of acetic acid than lactic acid was observed in the cassava leaves silage. The concomitant production of lactic acid and acetic acid increases the aerobic stability due to the inhibition of spoilage organisms (Danner *et al*., 2003). Moreover, Lindgren *et al*. (1990) reported that when the fresh material had low level of water soluble carbohydrate content or even no more available carbohydrate, LAB were able to utilize lactic acid and produce more acetic acid. The NH_3-N works as an important indicator of proteolytic activity during the fermentation process. The NH_3-N concentrations must not be higher than 11-12% of total nitrogen in well-preserved silages (Carpintero *et al*., 1969). Therefore, FJLB treatment of silage for 14 d can be considered excellent.

Conclusion

The results of this study showed that an increase of ensiling period could help better reduction of the HCNp. The results of both the experiments thus confirmed that using FJLB as additive to cassava silage would be one of the ways to reduce the HCNp content and also to increase the number of LAB in the finished product. It is suggested to use FJLB at the level of 1% (w/v) in the preparation of cassava silage to reduce HCNp content. According to the results of both experiments, this study also revealed that there is a negative correlation between pH decline and the rate HCNp reduction. These results demonstrate that low-cyanide cassava, which is safe to use as animal feed, could be produced by lactic acid fermentation using FJLB additive.

CHAPTER 4

Strategies for reducing of cyanogens in cassava and improving foliage and tuber yield by fertilizing

Abstract

The experiment was conducted to determine the effect of different fertilization rates on the chemical composition and yielding of cassava (foliage and tuber), in a one year experiment carried out in Okinawa, Japan. Treatments included nine rates each of nitrogen (N) and potassium (K) combination replicated thrice (N: 0, 50, 100; K: 0, 100, 250) in a complete randomized design. The suitable rates of fertilization were observed more pronounced effect on hydrocyanic acid potential (HCNp) reduction than the control treatment without N-K fertilizer application. The minimum HCNp values of foliage and tuber were obtained in F4 (N50-K100) and F5 (N50-K250) fertilization, although, there was no significant ($P>0.05$) different between them. The significantly highest ($P<0.05$) foliage and tuber yield were also observed in F4 and F5 fertilization treatments. The control plot produced lower crude protein (CP) content compared with other plots. In this study, N at 50 and K between 100 and 250 fertilization rates gave the higher cassava foliage and tuber yield with lower HCNp content than control was

observed. Thus, the F4 (N50-K100) was recommended for the optimum economical dose for the purpose of lower HCNp content of cassava for safe utilization.

Introduction

Cassava (*Manihot esculenta*, Crantz) is annual tuber crop grown widely in the tropical regions of the world. It is the fourth food crop after rice, wheat and maize on the list of major food crops in the developing countries (Cock, 1985). Focussing on the root production, many new high yielding varieties of cassava have been introduced, of which many have a high hydrocyanic acid potential (HCNp) content (Kim, 1999), and this leaf toxicity is a limiting factor in using a high level of cassava leaf in monogastric animals diets.

To achieve the yield potential of cassava, good soil fertility and adequate fertilization are essential (Gomez *et al*., 1980). The major nutrients required by cassava for optimum top growth and tuber yields are nitrogen (N) and potassium (K). Cassava plant is well adapted to low levels of available phosphorus (P) but requires fairly high levels of N and K, especially when grown for many years on the same plot or continuously cultivated plots (Ayoola and Makinde, 2007). Adequate K levels in soil stimulate response to N fertilizers but excess amount of both nutrients leads to

luxuriant growth at the expense of tuber formation (Onwueme and Charles, 1994). Many researchers reported that application of K increases starch content and decreases HCNp level (Obigbesan, 1973; Howler, 1985). On the other hand, application of increases N level, progressively increase the HCNp content (Sher *et al*., 2012). Hence, the need to upgrade the existing fertilizer recommendations in the sustainable cassava production is imperative. However, suitable fertilizer use with minimum polluting effects on the environment should be the major rule. For most crops, the best fertilizer types, rates and time of application were not known and this constituted a major constraint to fertilizer use in the country (Sarfo *et al*., 1998). Several studies have documented the proximate composition, amino acid profile (Rogers and Milner, 1963), and mineral content of cassava foliage (Ravindran *et al*., 1982), but in none was the HCNp content elucidated in relation to the different rates of fertilizer (N-K). There are few published reports that focus on agronomic management or cultivation practices for optimizing cassava foliage together with tuber production. Therefore, the objectives of the present study were to evaluate the HCNp and CP contents under nine rates of fertilizers and establish the yielding of foliage and tuber of cassava.

Materials and Methods

The experiment was carried out from July 2011 to Jun 2012 in the experimental field of the University of the Ryukyus, Okinawa, Japan. Figure 4.1 showed the climate data of experimental period which were obtained from Japan Meteorological Agency. Pre-treatment soil samples for soil analysis were taken before land preparation and fertilizer treatment. The soil fertility status before the commencement of experiment was shown in Table 4.1. The type of soil in the experimental area is gray soil (local named Jagaru).

The variety of cassava used in this experiment was a local variety called Red cassava, due to the red colour of the petiole. Old cassava stems, which were obtained after cutting into about 20 cm were planted in continuous rows with 50 cm between rows, 90 cm between stalks in the same row. Three levels of nitrogen and potassium fertilization (N-K_2O) were applied at three nitrogen rates of 0, 50 and 100 kg per hectare and three potassium rates of 0, 100 and 250 per hectare, composing a 3 x 3 factorial arrangement, were applied as a basal fertilizer. Single super phosphate (P_2O_5) was applied as a uniform rate 50 kg/ha for all treatments. This fertilizer was applied at the time of first leaf appeared from cassava stand at one month after planting. Tubers were harvested at the age of 12 month and their weights taken per stand to determine

the yielding of cassava foliage and tuber.

1. Chemical analysis

The total N was determined using micro-Kjeldahl method, the available P by the Truog (1930) and exchangeable K, sodium (Na), calcium (Ca), magnesium (Mg) by the method of Peech *et al*. (1962). Determinations were made of dry matter (DM) and CP using the procedures described by AOAC (1985) for all treatments. Cassava tuber and leaf materials were analyzed for the measurement of HCNp contents using acid hydrolysis method (Bradbury *et al*., 1991; Haque and Bradbury, 2002).

2. Statistical analysis

The data obtained on the parameters studied were subjected to analysis of variance from General Linear Model procedures using the software package SPSS 16.0 for windows. The means were separated using Duncan's Multiple Range Test from the same software.

Results

The average annual rainfall in the two experimental years was 2428 mm with a peak in rainfall in August, while monthly mean temperature ranged from 14.9 to 29.1˚C, with a minimum in January and a maximum in July. The commencement of the

experiment in July was to ensure adequate temperature for cassava. The application of fertilizer significantly affected on the HCNp content of foliage and tuber in this experiment (Table 4.2). The minimum HCNp content in cassava foliage was obtained by the application of F4 followed by F5. However, the difference between F4 and F5 was non-significant in this experiment. The similar result was observed in the HCNp content of tuber by the application of F5 followed by F4 at the final harvesting time of tuber. Moreover, the application of N-K treatments in tuber had significantly lower ($P<0.05$) HCNp content than those of the control treatment. Therefore, it is evident that the lowest level of HCNp content was observed at F4 and F5 fertilization rates in both foliage and tuber. The HCNp reduction as influenced by N-K fertilization compared with the control treatment for foliage and tuber were also presented in Table 4.2.

The CP yields in foliage and tuberous roots of cassava were summarized in Table 4.3 and 4.4, respectively. The high CP yield (356.24-404.79 kg/ha) in the foliage were obtained in F4, F5 and F7 fertilization treatments, although, there was no significant difference among them. The high CP yield of tuber also observed for F4 and F5 fertilization treatments were not significantly different between them, but were all significantly higher than control treatment.

The results of the growth attributes showed that N-K application tended to

increase the plant height compared with F0 treatment (Table 4.3). The control treatment (F0) had a smaller number of leaves and branches per plant than fertilized treatments. Moreover, the treatments were applied with N-K showed a higher number of leaves per plant than other treatments and it was observed in F5 followed by F4. Application of N fertilizer together with K (F4, F5, F7 and F8) showed more pronounced effect on the foliage yield compared with the control treatment (Table 4.3). The similar results were also obtained in tuber, although, there was no significant difference ($P>0.05$) among them (Table 4.4). The foliage yield peaked at F4, but the tuber yields obtained at F5 in this experiment. Both foliage and tuber yields generally, increased in all treatments with increasing rates of fertilizer application, however, it tended to decrease with higher rate of N-K dose at F7 and F8. The effect of different nitrogen and potassium levels on HCNp content and DM yield of cassava were also presented in Figure 4.2a and 4.2b, respectively.

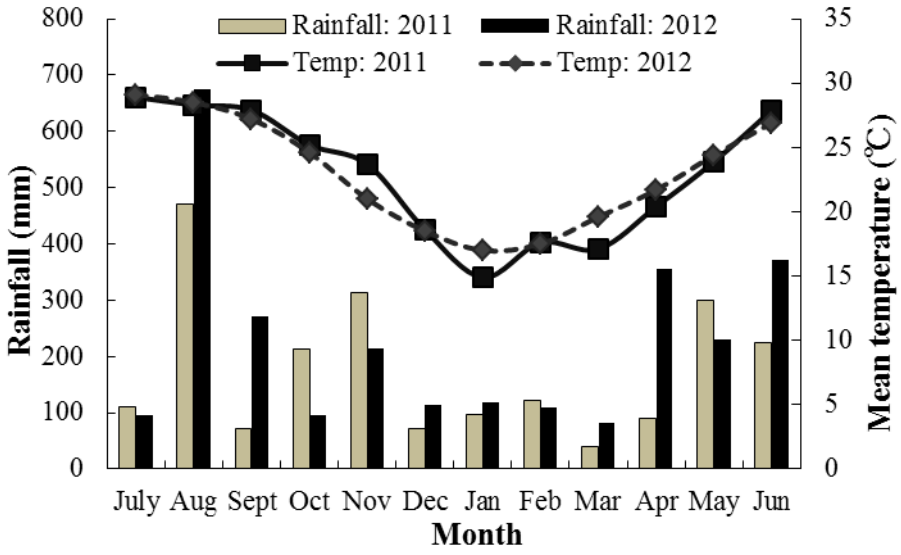


Figure 4.1 Average environmental temperature and rainfall in Okinawa (Naha) in 2011 and 2012.

Table 4.1 Soil nutrient composition for the 0-20 cm layer of the soil at the experimental site

Parameters	Values
pH (H_2O)	8.15±0.01
Total N (%)	0.14±0.01
Available P (ppm)	56.52±0.02
Exchangeable K (meq $100g^{-1}$)	0.26±0.02
Na (meq $100g^{-1}$)	0.24±0.01
Ca (meq $100g^{-1}$)	35.40±1.46
Mg (meq $100g^{-1}$)	1.97±0.33

Values are expressed as the mean ± SD.

Table 4.2 Effect of fertilizer application on HCNp content of cassava foliage and tuber

Fertilizer	Foliage		Tuber	
N-K_2O kg/ha	HCNp (mg/kg DM)	HCNp reduction (%)	HCNp (mg/kg DM)	HCNp reduction (%)
F0 (0-0)	129.49±9.06[a]	0.00±0.00[b]	95.66±8.03[a]	0.00±0.00[c]
F1 (0-100)	80.01±4.86[ab]	38.21±3.75[a]	65.55±6.97[b]	31.47±7.29[b]
F2 (0-250)	87.53±5.51[ab]	32.41±4.26[ab]	66.62±3.70[b]	30.35±3.87[b]
F3 (50-0)	76.82±7.38[ab]	40.67±5.70[a]	55.37±4.64[b]	42.11±4.84[b]
F4 (50-100)	51.89±4.84[b]	59.93±3.73[a]	34.02±4.95[c]	64.44±5.18[a]
F5 (50-250)	54.77±9.92[b]	57.70±7.66[a]	31.97±1.51[c]	66.58±1.57[a]
F6 (100-0)	86.43±2.25[ab]	33.25±1.74[ab]	59.11±9.93[b]	38.21±5.39[b]
F7 (100-100)	67.01±8.56[ab]	48.25±6.61[a]	54.94±3.16[b]	42.57±3.30[b]
F8 (100-250)	66.85±3.98[ab]	48.37±3.08[a]	50.95±4.80[b]	46.73±5.02[b]

[a-c] Values (mean ± SD) with different superscript on the same column are significantly different ($P<0.05$).

Table 4.3 Effect of fertilizer application on protein%, growth characteristics and yielding of cassava foliage

Fertilizer	Dry matter	Crude protein	Plant height	Leaf no.	Branches	Foliage yield	Protein yield
N-K_2O kg/ha	(%)	(% DM)	(cm)	per plant		(kg/ha DM)	
F0 (0-0)	25.81±0.60	12.54±0.60	149.60±4.81^{b}	229.33±14.74^{c}	7.67±0.57^{b}	1479.33±153.46^{d}	185.30±27.09^{d}
F1 (0-100)	25.70±2.99	13.20±0.78	166.13±14.28^{ab}	307.67±25.92^{abc}	9.00±1.73^{ab}	1821.83±167.34^{cd}	239.79±14.75^{bcd}
F2 (0-250)	25.54±0.68	13.25±0.94	160.07±11.15^{ab}	394.33±15.66^{ab}	12.67±2.08^{ab}	1810.43±149.95^{cd}	238.50±35.35^{bcd}
F3 (50-0)	24.79±3.51	13.64±0.59	156.67±11.95^{ab}	291.00±8.00^{bc}	13.00±2.65^{ab}	1494.13±122.37^{d}	204.07±13.48^{cd}
F4 (50-100)	24.99±1.61	13.80±0.48	183.60±8.35^{a}	395.00±13.00^{ab}	12.67±2.64^{ab}	2930.93±188.07^{a}	404.79±35.08^{a}
F5 (50-250)	25.58±0.36	13.82±0.05	182.20±1.50^{a}	435.00±16.27^{a}	13.00±2.65^{ab}	2684.73±159.33^{a}	370.87±22.76^{a}
F6 (100-0)	25.96±1.47	13.58±0.35	162.87±8.88^{ab}	299.00±7.91^{abc}	13.00±2.65^{ab}	2102.23±99.82^{bc}	285.71±15.88^{b}
F7 (100-100)	27.16±1.57	14.09±1.49	164.47±2.65^{ab}	363.67±13.98^{abc}	14.33±2.08^{a}	2528.53±170.76^{ab}	356.24±26.05^{a}
F8 (100-250)	24.87±0.60	13.96±0.85	165.07±2.75^{ab}	336.00±12.41^{abc}	12.33±1.15^{ab}	1877.73±187.61^{cd}	260.78±15.98^{bc}

$^{a-d}$ Values (mean ± SD) with different superscript on the same column are significantly different (P<0.05).

Table 4.4 Effect of fertilizer application on CP% and yielding of cassava tuber

Fertilizer	Dry matter	Crude protein	Tuber yield	Protein yield
N-K_2O kg/ha	(%)	(% DM)	(kg/ha DM)	
F0 (0-0)	23.18±1.98	0.87±0.08[b]	4639.20±912.60[b]	40.82±10.26[c]
F1 (0-100)	25.67±0.57	1.00±0.02[ab]	6115.80±895.88[ab]	61.12±9.88[ab]
F2 (0-250)	25.14±1.65	0.99±0.02[ab]	7229.50±911.56[ab]	71.60±11.25[abc]
F3 (50-0)	26.06±1.62	1.13±0.10[ab]	6401.40±649.79[ab]	73.35±18.48[abc]
F4 (50-100)	26.55±2.38	1.25±0.06[a]	9124.40±883.79[a]	114.07±13.36[a]
F5 (50-250)	25.07±0.71	1.19±0.16[ab]	9474.10±822.20[a]	114.62±17.70[a]
F6 (100-0)	25.39±0.55	1.28±0.06[a]	6363.70±781.13[ab]	81.91±15.05[abc]
F7 (100-100)	26.32±1.20	1.24±0.07[a]	7766.00±812.43[ab]	96.59±17.03[ab]
F8 (100-250)	25.75±1.74	1.32±0.21[a]	6897.60±955.33[ab]	85.90±16.61[ab]

[a-c] Values (mean ± SD) with different superscript on the same column are significantly different ($P<0.05$).

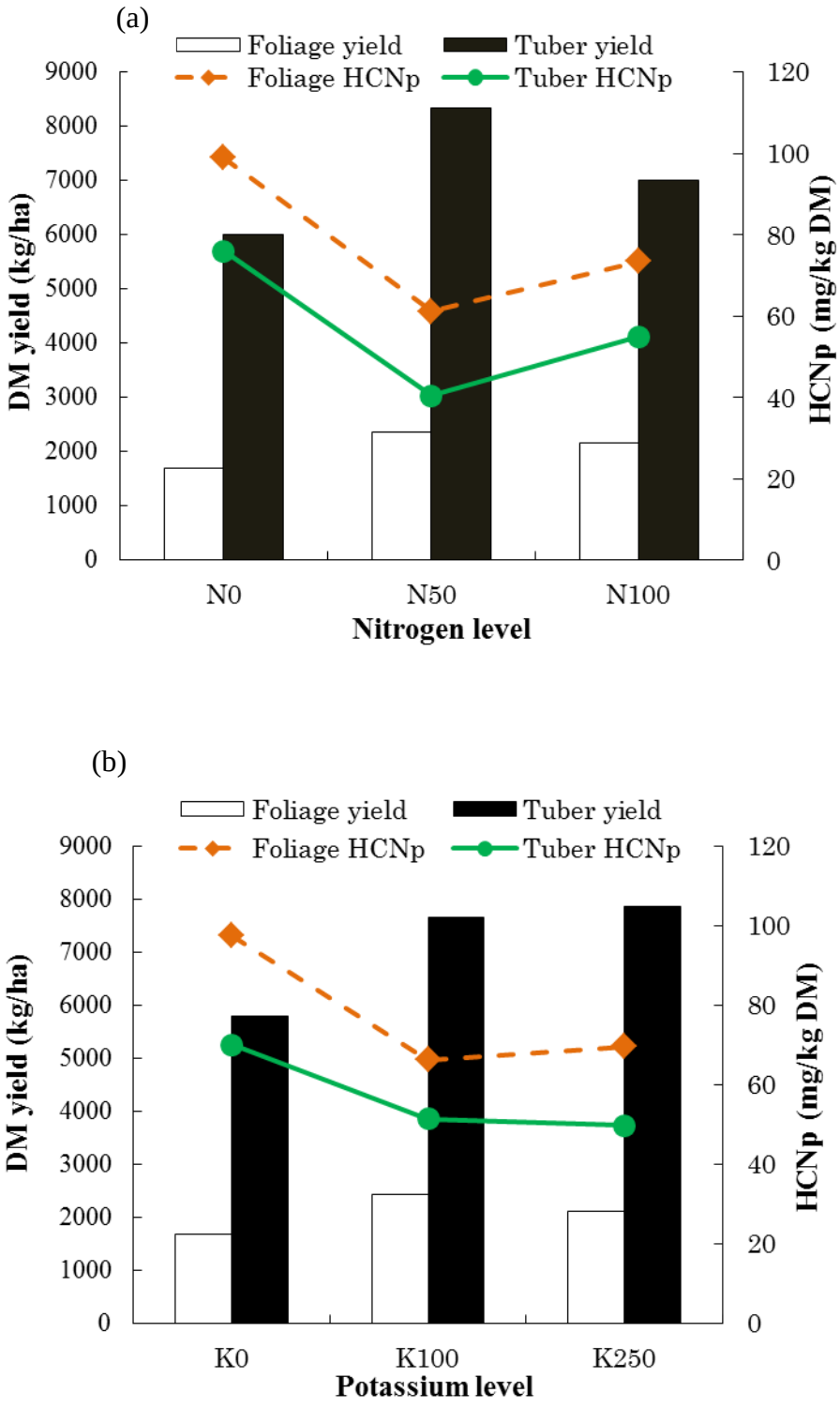


Figure 4.2. Effect of different nitrogen levels (a) and potassium levels (b) on HCNp content and DM yield of cassava.

Discussion

The average values of HCNp content in the present results are included in the range of low HCNp levels compared to the recent report of Hue *et al*. (2012). The amount of HCNp content in cassava varies even different parts of the same plant according to plant age and other factors like soil and fertilization contribute to the quantities of HCNp in the plants (Bradbury *et al*., 1999). In this experiment, higher rate of N application tended to increase in HCNp content and it was observed in F6, F7 and F8 compared with F4 and F5 treatments. Therefore, it could be reasoned that the suitable quantity of N fertilizer application would encourage plant growth to the climax, but the excess dose would enhance to increase the HCNp content. Sher *et al*. (2012) also revealed that increase in N application resulted in enhanced HCNp level. Furthermore, Peter and Birger (2002) stated that the applied N stimulates the enzymatic conversion of tyrosine to p-hydroxymandelonitrile which ultimately lead to increase in the biosynthesis of cyanogenic glucoside. Worthington (2001) also stated that plants require N for normal growth and protein synthesis however, if N is applied in excess of what the plant requires for protein formation, the excess is accumulated as nitrate and stored predominantly in the green leaf part of the plant. The highest value of HCNp reduction was observed in F4 and F5 in this experiment. Therefore, the

appropriate combination rate of N and K are required for cassava cultivation concerning for the HCNp reduction. The same trend of HCNp reduction as influenced by fertilization was observed in tuber. From this result, the combined N-K treatment showed a higher HCNp reduction than the individual treatment of either N or K. Therefore, the HCNp content in tuber clearly showed that both N-K application promote the HCNp reduction. Moreover, the highest HCNp reduction with the minimum HCNp content in tuber were observed in the higher dose of K namely F5 followed by F4 in this experiment. Therefore, the results obtained in this experiment are consistent with the report of Howeler (2002). Putthacharoen *et al.* (1998) who stated that cassava removed less N and P but similar amounts of K in the harvested plant parts as compared to maize, sorghum, peanut, mungbean, pineapple and sugarcane. Long-term fertility trials indicate that without adequate K fertilizer, in this case referring to tuber production, cassava yields eventually decline due to K depletion, except in those soils containing large amounts of K-bearing minerals (Howeler, 1991).

In this experiment, the control treatment without N and K fertilization produced lower CP content compared with other treatments. Ravindran (1993) reported that the foliage contains approximately 21% CP with a range from 17 to 40% CP depending on cultivar, maturity, sampling procedure, soil fertility and climate. Nitrogen increased the

chlorophyll of leaves thereby promoting the photosynthetic capacity of the plant, plays a part in the manufacture of proteins and is also responsible for high yield in plants. The CP yields of foliage and tuber generally, increased in all treatments as compared to control with increasing rates of N fertilizer application in this experiment. The increase in protein content with N fertilization is in agreement with the finding of Mahmud *et al.* (2003). Potassium on the other hand, promotes CO_2 assimilation and translocation of carbohydrates from leaves to the tubers and tuberous roots of crops where carbohydrates are the main storage material (Howeler, 2002).

The control plots recorded the shortest plants in height with the lowest number of leaves and branches. The superior growth attributes obtained by application of N and K in this experiment had been reported by Uwah *et al*. (2013). The positive response of growth characters to the applied nutrients is suggested to attributable to their role in cell multiplication and photosynthesis which gave rise to increase in size and length of leaves and stems. Nitrogen is a major element (Mosier *et al*., 2004) that is essential for synthesis of amino acids, nucleic acids and some organic acids which is necessary for plant growth and development and its limits reduce yield (Zhao *et al*., 2005). Okpara *et al*. (2010) reported that plant height was increased by application of K up to 150 kg/ha.

Fertilization resulted in higher foliage and tuber yields in the fertilized plots than

the control. This observation supports the findings of Gomez *et al*. (1980) who obtained higher cassava yield when fertilizer was applied. Molina and EI-Sharkawy (1995) also reported that fertilization induced production of more vigorous plants, increased nutrient recycling from fallen leaves and improved the quality of the planting material. The increase in fodder yield with fertilizer application may be due to greater plant height, higher stem diameter, higher number of leaves per plant and greater leaf area per plant (Mahmud *et al*., 2003). Hence, Mehdi *et al*. (2007) stated that the positive response of tuber yield and yield components to increased rates of N and K could be adduced to high starch synthesis and translocation activities stimulated by N and K application.

Parkes *et al*. (2012) reported that tuber yields generally increased in all cassava genotypes with increasing rates of fertilizer application up to 120N-180K_2O kg/ha. However, they recommended that the economic rate of the fertilizer application for all genotypes was 60N-90K. Viewing both yield and HCNp contents, the results of present experiment exhibited that F4 and F5 fertilizer combinations gave the higher cassava yield with lower HCNp content than without N-K fertilization. Furthermore, the maximum dry matter yield and lowest HCNp content in foliage and tuber was obtained by the application of N at 50 and K between 100 and 250 fertilization rates. Therefore,

the combination of N50 and K100 fertilization appeared appropriate for optimum yield and HCNp reduction in our study.

Bolhuis (1954) had set the following classification of toxicity according to HCNp content: 0-50 mg/kg, innocuous or harmless, 50-100 mg/kg moderately toxic and >100 mg/kg dangerous or toxic. Therefore, this study revealed that the control treatment had the HCNp level (129.49 mg/kg) considered poisonous level while other treatments were moderately poisonous level (51.89-90.00 mg/kg).

Conclusion

Present results clearly indicated that content of cyanide poisoning can be controlled by appropriate fertilization management. The combination ratio of nitrogen and potassium (50 kg/ha and 100 kg/ha) was recommended as the optimum economical dose for the purpose of lowering HCNp content of cassava for safe utilization as feed.

CHAPTER 5

Effects of harvesting period on chemical composition and yielding of cassava foliage and tuber

Abstract

The experiment was conducted to determine the effect of different harvesting period on the chemical composition and yielding of cassava (foliage and tuber). Two different ages of cassava foliage of initial harvesting, at 3 and 5 months were conducted for IH3 + FH and IH5 + FH treatments, respectively. The final harvests of these two treatments were done the whole including tuber in the 7 month. Cassava foliage, harvested once at root harvest (7 month), was performed as a control treatment (FH). The lowest hydrocyanic acid potential (HCNp) content was observed at control treatment in both cassava foliage and tuber while IH3 + FH treatment showed the highest HCNp content. The HCNp and crude protein (CP) content were higher in the leave compared with petiole and stem of foliage while these compositions were also higher in cortex than parenchyma portion of cassava tuber. The opposite trend was found in the crude fiber (CF) contents, which were higher in the petiole and stem than the leaves. The highest total foliage and protein yield were observed at IH5 + FH

treatment compared with IH3 + FH and FH. FH treatment produced the highest tuber yield (15268 kg/ha), followed by IH5 + FH (11567 kg/ha) in this experiment. The leaves and tuber contain gross energy with an average of 4709 kcal/kg (range: 4608-4783 kcal/kg) and 3857 kcal/kg (range: 3842-3881 kcal/kg), respectively. Viewing both yield and proximate analytical values, the IH5 + FH treatment gives the highest foliage yield together with the high CP and low fiber content in both harvest periods.

Introduction

Cassava foliage has been reported to have good amino acid content, comparable to soybean meal (Eggum, 1970) and the amount of foliage available at root harvesting is equivalent to about 30% of the tuber yield (Ravindran, 1993). Being a very important animal feed and industrial raw material, cassava are used for dual purpose nowadays; not only for tuber but also for foliage production. Several studies have been undertaken on the use of cassava leaves as feed for cattle (Moore and Cock, 1985), goats (Seng and Rodriguez, 2001), pigs (Ravindran *et al*., 1987) and poultry (Ravindran *et al*., 1986). However, cassava leaves evaluated in much of the earlier research were mainly from the plant after harvesting the tubers and these probably

have a higher fiber content due to their maturity. The use of its by-products in animal feeding could be an alternative for both the farmer that subsists on this crop and for the industry. In addition, with proper management practices, it is expected that both tuber and foliage yields will increase and the sustainability of the system will improve.

Up to the present time, there are few papers describing the behavior of nutritional status as influenced by different initial stages of cutting and subsequent harvesting interval as well as its relation to the foliage yield (Hong *et al*., 2003) and changes in the nutritional composition of leaves during maturity (Ravindran and Ravindran, 1988). No data on the nutritional status and HCNp content by the effect of harvesting period and its effects on foliage and tuber production were reported. Therefore, in this study, it was considered of interest to focus on leaves harvested at a younger stage with appropriate nutrient inputs as well as tubers under these conditions.

Materials and Methods

The experiment was carried out from April 2013 to October 2013 in the field of the University of the Ryukyus, Okinawa, Japan. Climatic data recorded during the experimental period was showed in Figure 5.1 which was obtained from Japan Meteorological Agency. It also presents 23.6˚C mean annual temperature; 2071 mm

mean annual rainfall during the experimental period. The chemical analysis of soil samples before setting up the experiment, was showed in Table 5.1. The type of soil in the experimental area is gray soil (local named Jagaru). Randomized complete design (RCD) with three replications was used to study the effects of different harvestings on chemical compositions and yielding of cassava foliage and tuber. The experiment comprised of 3 treatments:

IH3 + FH; Top harvesting in the 3 months and final harvest

IH5 + FH; Top harvesting in the 5 months and final harvest

FH; Final harvest including tuber in the 7 months only as a control treatment

The variety of cassava used in this experiment was a local one called Red cassava, due to the red colour of the petiole. Old cassava stems, which were obtained after cutting into about 20 cm, were planted in continuous rows with 1 m between rows, 1 m between plants to give a plant population of 7500 stands/ha. Nitrogen, phosphorus and potassium (N-P_2O_5- K_2O) were applied at the rate of 50, 50 and 100 kg per hectare as a basal fertilizer. This fertilizer was applied at the time of first leaf appeared from cassava stand at one month after plating. The harvesting of cassava foliage was done according to treatments. The last harvesting of cassava foliage for all treatments was done when harvesting cassava tuber at 7 month after planting. Twenty-seven plants for

each treatment in respective harvests were randomly selected and cassava foliage was harvested by breaking the stem at between the green and brown part in accordance with Wanapat *et al*. (1997). Individual plot of fresh cassava foliage were weighed. The cassava foliage samples (leave, petiole and stem) were randomly sampled and divided into two parts, one for HCNp content and one for other chemical analyze. Cassava tuber was harvested at 7 month after planting, soil-free cassava tuber in each plot was weighed to calculate yielding. Cassava tuber were separated into cortex and parenchyma and subjected to chemical analysis.

1. Chemical analysis

The total N was determined using micro-Kjeldahl method, the available P by Truog (1930) and exchangeable K, sodium (Na), calcium (Ca), magnesium (Mg) by using Inductively Coupled Plasma atomic Emission spectroscopy (ICPE-9000, Shimadzu, Japan). The gross energy was determined by using Auto-calculating Bomb Calorimeter (CA-4AJ, Shimadzu Co. Ltd., Japan). Dry matter (DM) content of experimental material was determined by oven drying at 70˚C to a constant weight. Determinations were made of CP and CF using the procedures described by AOAC (1985). Cassava tuber and leaf materials were analyzed for the measurement of HCNp contents using acid hydrolysis method (Bradbury *et al*., 1991; Haque and Bradbury,

2002).

2. Statistical analysis

The statistical analysis of the data, obtained at each stage of the study was performed using the SPSS 16.0 Software by ANOVA in accordance with the General Linear Model. The Duncan new multiple range test was used to assess significant differences among treatments.

Results

The mean HCNp contents in the different parts of cassava foliage for all harvesting treatments are shown in Table 5.2. The significant highest ($P<0.05$) HCNp content occurred at the first harvest of IH3 + FH treatment, at 3 month of age. From this period onwards, a continuous decrease in the contents was recorded until the last harvest at 7 month in this treatment. However, the HCNp content in cassava leaves of IH5 + FH treatment was tended to increase from first harvest (152.18 mg/kg) to second harvest (188.44 mg/kg). The lowest HCNp content of cassava foliage was recorded in the control treatment. The HCNp contents in cassava foliage were in the range leave>petiole>stem in all harvesting treatments. Similar observations have been reported by De Bruijn (1973). The opposite trend was found for the CF contents, which

were higher in the petiole and stem than the leaves (Table 5.2). The similar CF contents were observed between petiole and stem range from 19.97 to 28.01% in all treatments. The HCNp content in cortex were not significantly difference ($P>0.05$) among treatments but were significantly higher ($P<0.05$) than parenchyma in all harvesting treatments (Table 5.3). There was significant difference ($P<0.05$) between the CF contents in different parts of cassava tuber, as the fiber contents in the cortex were higher than in the parenchyma.

As shown in Table 5.4, the CP content in cassava foliage was significantly higher at the first harvest of IH3 + FH treatment, and then decreased in the following foliage harvest. Cassava leaves also had a higher content of CP than petiole and stem. The significant higher ($P<0.05$) CP contents were recorded in the cortex portion than parenchyma of cassava tuber. Leaf, petiole and stem ratios of the cassava foliage in all harvests were shown in Table 5.5. Leaf accounted for a higher proportion than petiole and stem of the whole crop. There were significant differences in the proportion of leaf, petiole and stem between treatments. The lowest petiole and stem ratio was observed in the 7 month harvest of IH5 + FH among all final harvest period treatments.

Figure 5.2a also indicated that total foliage yield and protein yield of cassava. The significant highest ($P<0.05$) total foliage yield was observed at IH5 + FH (3721.73

kg/ha) compared with IH3 + FH (3209.48 kg/ha) and FH (3426.90 kg/ha). Therefore, it corresponds to give the highest CP yields with the values 1878.54 kg/ha in this IH5 + FH treatment while 1733.92 kg/ha and 803.61 kg/ha were obtained in the treatments of IH3 + FH and FH, respectively. The DM yields of tuberous roots among the 3 treatments were also shown in Figure 5.2b. In these treatments, the DM tuber yield was highest in control treatment (15268 kg/ha) but lowest in IH3 + FH treatment (8823 kg/ha). As a consequence with the tuber yields, the CP yield was observed with the same trends in this experiment. The effect of different harvesting periods on gross energy values of cassava foliage and tuber were also presented in Table 5.6.

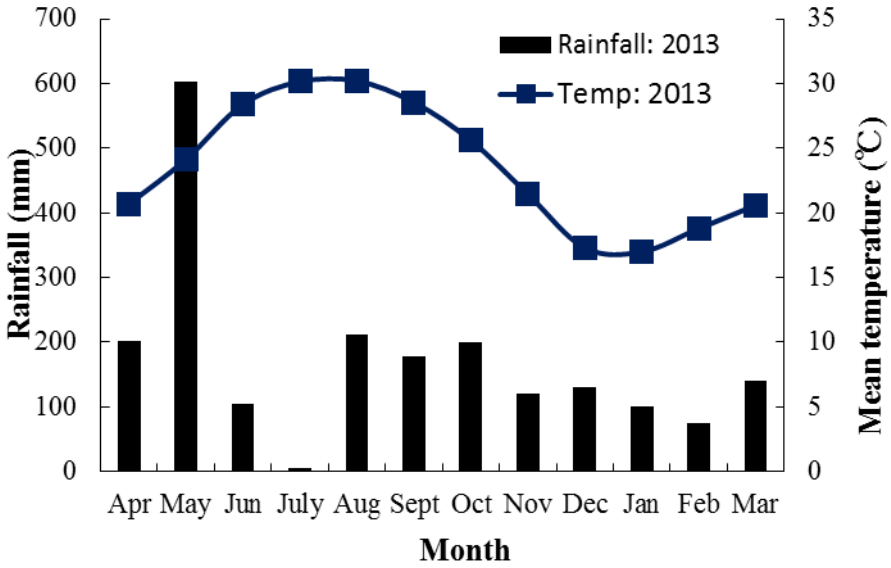


Figure 5.1 Average environmental temperature and rainfall in Okinawa (Naha) in 2013.

Table 5.1 Soil nutrient composition in the 0-20 cm layer of the soil at the experimental site

Parameters	Values
pH (H_2O)	7.86±0.01
Total N (%)	0.14±0.01
Available P (mg $100g^{-1}$)	15.04±3.71
Exchangeable K (meq $100g^{-1}$)	1.11±0.14
Na (meq $100g^{-1}$)	0.74±0.15
Ca (meq $100g^{-1}$)	59.87±2.15
Mg (meq $100g^{-1}$)	2.39±0.14

Values are expressed as the mean ± SD.

Table 5.2 Effect of harvesting period on HCNp and fiber contents of cassava foliage

Item	Parts	IH3 + FH		IH5 + FH		FH
		Month 3	Month 7	Month 5	Month 7	Month 7
HCNp	Leaf	237.76 ± 10.30^{Aa}	163.29 ± 7.78^{Ac}	152.18 ± 5.72^{Ad}	188.44 ± 4.26^{Ab}	117.94 ± 10.55^{Ae}
(mg/kg DM)	Petiole	168.19 ± 8.91^{Bb}	146.93 ± 7.12^{Bc}	150.73 ± 2.74^{Ac}	187.57 ± 6.11^{Aa}	101.68 ± 10.37^{Bd}
	Stem	150.98 ± 9.34^{Ca}	145.79 ± 6.14^{Ba}	145.03 ± 2.54^{Ba}	144.78 ± 7.45^{Ba}	90.88 ± 11.1^{Bb}
CF	Leaf	6.43 ± 1.16^{Cc}	11.35 ± 0.05^{Ba}	10.31 ± 0.26^{Bb}	9.67 ± 0.35^{Cb}	11.84 ± 0.95^{Ba}
(%)	Petiole	19.97 ± 3.88^{Bb}	28.01 ± 0.75^{Aa}	26.48 ± 1.73^{Aa}	27.91 ± 0.33^{Aa}	26.67 ± 1.96^{Aa}
	Stem	23.77 ± 1.99^{Ab}	27.21 ± 2.12^{Aa}	27.24 ± 0.15^{Aa}	26.91 ± 0.38^{Ba}	27.50 ± 0.43^{Aa}

$^{A-C}$Values (mean ± SD) with different superscript on the same column are significantly different ($P<0.05$).
$^{a-e}$Values (mean ± SD) with different superscript on the same row are significantly different ($P<0.05$).

IH3 + FH; Top harvesting in the 3 months and final harvest
IH5 + FH; Top harvesting in the 5 months and final harvest
FH; Final harvest including tuber in the 7 months only as a control treatment

Table 5.3 Effect of harvesting period on HCNp and fiber contents of cassava tuber

Item	Parts	Harvesting treatments		
		IH3 + FH	IH5 + FH	FH
HCNp	Cortex	160.53±5.79^{A}	151.66±2.05^{A}	152.25±2.44^{A}
(mg/kg DM)	Parenchyma	89.69±6.54^{Bab}	93.49±2.67^{Ba}	82.43±4.49^{Bb}
CF	Cortex	6.37±1.38^{A}	6.66±1.31^{A}	7.33±0.76^{A}
(%)	Parenchyma	0.80±0.27^{Bb}	1.30±0.18^{Ba}	1.43±0.33^{Ba}

$^{A-B}$Values (mean ± SD) with different superscript on the same column are significantly different (P<0.05).
$^{a-b}$Values (mean ± SD) with different superscript on the same row are significantly different (P<0.05).
IH3 + FH, IH5 + FH and FH referred to Table 5.2.

Table 5.4 Effect of harvesting period on crude protein % of cassava foliage and tuber

	Parts	IH3 + FH		Total	IH5 + FH		Total	FH
		Month 3	Month 7		Month 5	Month 7		Month 7
Foliage	Leaf	30.65±1.42^{Aa}	23.37±0.62^{Ac}	54.03	25.17±0.25^{Ab}	25.31±1.58^{Ab}	50.48	23.45±0.33^{Ac}
	Petiole	9.56±1.64^{Ca}	5.55±0.75^{Bb}	15.11	5.30±0.18^{Cb}	5.47±0.15^{Bb}	10.77	5.44±0.22^{Cb}
	Stem	14.16±2.68^{Ba}	5.94±0.40^{Bd}	20.09	10.42±1.11^{Bb}	6.07±0.21^{Bd}	16.49	7.81±0.63^{Bc}
		IH3 + FH			IH5 + FH			FH
Tuber	Cortex	5.07±0.17^{Ab}			5.98±0.74^{Aa}			6.55±0.58^{Aa}
	Parenchyma	1.94±0.26^{B}			2.30±0.29^{B}			2.22±0.27^{B}

$^{A-C}$Values (mean ± SD) with different superscript on the same column are significantly different (P<0.05).

$^{a-d}$Values (mean ± SD) with different superscript on the same row are significantly different (P<0.05).

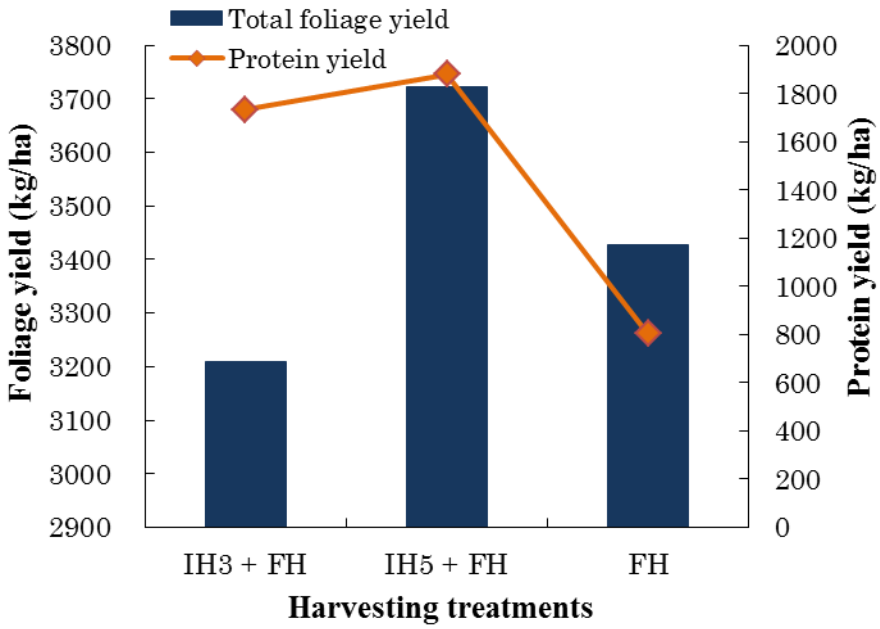
IH3 + FH, IH5 + FH and FH referred to Table 5.2.

Table 5.5 Proportion of cassava foliage on each harvesting treatments

Proportion	IH3 + FH		IH5 + FH		FH
(%)	Month 3	Month 7	Month 5	Month 7	Month 7
Leaf	88.50 ± 0.82^{Aa}	54.61 ± 4.11^{Ad}	61.90 ± 1.91^{Ab}	62.94 ± 1.66^{Ab}	56.21 ± 1.30^{Ac}
Petiole	7.82 ± 0.77^{Bd}	23.61 ± 5.37^{Ba}	17.99 ± 1.01^{Cc}	18.87 ± 1.46^{Bc}	20.99 ± 0.85^{Cb}
Stem	3.67 ± 0.41^{Ce}	21.78 ± 1.71^{Bb}	20.11 ± 0.96^{Bc}	18.19 ± 1.15^{Bd}	22.81 ± 0.55^{Ba}

$^{A-C}$Values (mean ± SD) with different superscript within each column are significantly different at $P<0.05$.
$^{a-e}$Values (mean ± SD) with different superscript within each row are significantly different at $P<0.05$.
IH3 + FH, IH5 + FH and FH referred to Table 5.2.

(a)



(b)

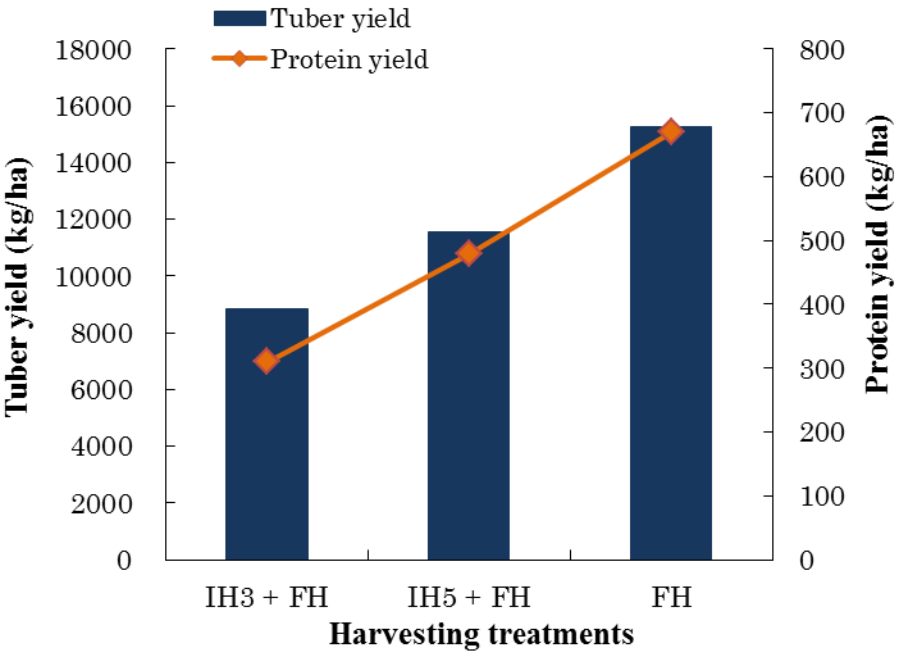


Figure 5.2. Effect of harvesting period on DM yield and protein yield of cassava foliage (a) and cassava tuber (b).

IH3 + FH, IH5 + FH and FH referred to Table 5.2.

Table 5.6 Effect of harvesting periods on gross energy values of cassava foliage and tuber

		Harvesting treatments		
		IH3 + FH	IH5 + FH	FH
Gross energy (kcal/kg)	Foliage	4608.13±83.34	4736.87±25.44	4782.57±83.63
	Tuber	3841.67±15.77	3881.08±21.14	3849.08±22.95

Values (mean ± SD) were not significantly different at $P > 0.05$.

IH3 + FH, IH5 + FH and FH referred to Table 5.2.

Discussion

Different harvesting treatments had a considerable effect on the HCNp content of the foliage examined in the present study. Sundaresan *et al*. (1987) reported that the HCNp concentration and the bitterness associated with high cyanogenic glycoside contents in leaves decreases with the maturity of the leaves. Therefore, the HCNp content in IH3 + FH treatment was tending to decrease at the final harvest. However, the increased in HCNp content in the IH5 + FH treatment showed that the foliage could be produced with high level of HCNp content if the short period of regrowth was allowed. It could be assumed that the IH3 + FH treatment had 4 months for regrowth period after first harvest and this period allowed the leaf to mature state. On the other hand, the harvest interval at IH5 + FH was only 2 months for regrowth and it would produce higher HCNp content in a younger state of the leaves. Moreover, the results of this study (Table 5.2) showed higher HCNp contents in leaves compared to other parts of the cassava crop and agreed with the findings of Etonihu *et al*. (2011). In addition, it was reported that the leaves also contained the enzyme hydroxynitrile lyase, which catalysis the hydrolysis of acetone cyanohydrin to produce HCN and acetone (Siritunga *et al*., 2004).

Cassava tubers showed slightly variation in HCNp content under the condition of

different foliage harvesting period. According to Vetter (2000), cyanogenic glycosides are mainly synthesized in the cotyledons of etiolated seedlings or in the young green leaves of mature plants and transported to other part of the plants, such as tuberous root. The concentration of HCNp contents in the foliage of IH3 + FH and IH5 + FH treatment could be probably due to transport the higher concentration of HCNp from the foliage to the tuber than control treatment. Therefore, the lowest total HCNp content was observed in the tuber of control treatment. Although translocation of linamarin takes place from leaves to tuber, there appears to be no progressive accumulation of linamarin in tuber, indicating that linamarin is not passively stored in the tuber, but is metabolized and utilized (Makame *et al*., 1987).

Etonihu *et al*. (2011) reported that the cortex of tuber is the outermost layer part that is mostly exposed to different biochemical nutrient in the soil. As a result, exposure to hydrocyanide in the soil could lead to increase proportion of cyanide in part of the plant. Therefore, the profile of HCNp concentration in this study showed that tubers (parenchyma) are lower cyanide storage part of cassava than cortex and explains the reason for the higher consumption of this part by man and animals.

The mean CF content in the leaves of the final harvest of all treatments was with a range of 9.67 to 11.84% (Table 5.2). These values of fiber in this study were lower

than those reported by IITA (1990). It is possible that the differences were probably due to differences in harvesting time which findings are also in agreement with Phengvilaysouk and Wanapat (2008). Furthermore, the fiber content in IH5 + FH treatment was lower than other harvest treatments. The fodder having less CF percentage is considered as a good quality because high dietary fiber can cause intestinal irritation, lower digestibility and overall decreased nutrient utilization (Almodares *et al*., 2009). In addition, the cortex portion of cassava tuber in the control treatment was showed higher fiber content than the other treatments. Therefore, the subsequent harvesting of foliage slightly affected to the fiber content of tuber. Tewe (2004) pointed out that the use of cassava peel as feed for non-ruminant animals is limited due to its high fiber content and HCNp which are deleterious to their growth and development.

As the results, in general, the CP content in the young leaves was higher than that in the old leaves. According to Gomez and Valdivieso (1985), the CP and fiber content are the two chemical components that are mainly affected by increasing plant age, with CP decreasing and fiber content increasing as the biomass becomes older. Furthermore, cassava leaves have been reported as having higher protein content than stems and petioles (Borin, 2005), and therefore, cassava leaves will be suitable for monogastric

animals such pigs and poultry, while stem plus petioles or whole plant are more suitable for ruminants.

The total DM yield of cassava foliage in the present study (3209 to 3721 kg/ha) was in the range reported by Wanapat (2002), who found that the total DM yield of foliage can vary from 1814 to 7257 kg/ha. Gomez and Valdivieso (1984) described that the potential yield of cassava leaves or foliage varies considerably, depending on variety, age of plants, plant density, soil fertility, harvesting frequency and climate. Khajarern and Khajarern (1992) pointed out that leaves can be harvested within 4 to 5 months of planting, without adversely affecting tuber production. This assumption is confirmed by the fact that the yield at the 5 month harvest was highest that this harvest took place after a period of high growth rate of leaves and stems.

Leaf stem ratio is important in the study of the protein concentration of above-ground parts of the plant, because leaves have the highest amino acid concentrations in cassava (Normanha, 1966 cited in De Pinho *et al*., 2004). The results from this study also showed that leaf accounted for a higher proportion than petiole and stem of the whole crop. The mean dry leaf proportion of the foliage was high (65%) but with a wide range, from 54 to 88% (Table 5.4). These findings were also supported by work of Wanapat (2002) who found that cassava hay harvested at 4

month planting had a leaf proportion of 62%. However, the mean value was slightly higher than the results reported by Meyrelles *et al*. (1977) where the leaf proportion of cassava foliage on DM basis was almost 52%. In general, leaves of the cassava constitute a very significant proportion of the whole plant, making cassava fodder a valuable feed material for animal feed.

Tuberization in cassava, which occurs as a result of swelling of the fibrous roots, can commence within 30-60 days (Cock *et al*., 1979). If the cassava is cultivated primarily for its tubers, it is imperative that leaf harvesting should not greatly reduce tuber yield. Jalloh (1998) showed that delaying the first foliage collection until the 4^{th} month allows the plant to pass the most critical stage for its tuberous root yield. Therefore, Lockard *et al*. (1985) recommended that the time at which leaf collection was started on cassava had little effect on the total weight of leaves and the weight of the tuberous roots, so leaf collection can start at least as early as 4 months after planting. Gross energy values averaged 4709 kcal/kg in leaves and 3857 kcal/kg in tuber were observed in our study. Okeke (1980) reported that cassava is high energy yielding and continuously available plant. Furthermore, cassava is capable of providing higher amount of energy/ha, about 13 times more than maize or guinea corn (Sorghum) (Oyenuga 1961). Therefore, Tewe (1997) stated that the usage of cassava as

an alternative to conventional energy feedstuffs like maize could help reduce cost of feed and alleviate the problem of direct competition between livestock and humans for maize. According to the present results, the cassava crop could be cultivated for the production of foliage but at the expense of tuber yield, so that foliage and tuber production cannot be achieved simultaneously.

Conclusion

The study revealed that the greatest advantage of two times harvest (IH5 + FH) is that forage can be obtained the highest foliage yield with the acceptable level of tuber yield. Therefore, it is suggested to harvest at the initial harvesting at 5 months and final harvest system because enough protein source including young leaves was obtained.

CHAPTER 6

Effects of cassava substitute for maize based diets on performance characteristics and egg quality of laying hens

Abstract

This experiment was conducted to evaluate the efficiency of feeding diets containing cassava meal (tuber and leaf) on feed intake, feed conversion ratio, weight gain, egg performance and egg quality (weight, shell quality, yolk color, egg yolk cholesterol contents) of laying hens during 5 weeks of feeding experiment. Thirty-six white leghorn laying hens were allocated into 4 groups of nine hens each. Diet I, contained no cassava meal but maize 57% of the diet and served as the control. In diet II, III and IV, the proportion of maize was substituted with the cassava meal at the levels of 30% (30% tuber + 0% leaf), 40% (30% tuber + 10% leaf), and 50% (30% tuber + 20% leaf), respectively. Cassava meal including tuber and leaf did not have significant dietary effects on the performance of laying hens. However, egg laying rate tended to decrease with the IV, although there was no significant different between them. Egg quality parameters including Haugh unit value in the hens fed diet III were significantly higher ($P<0.05$) than those fed diet I, II and IV. Hens fed with

diets containing cassava either tuber or leaf had higher ($P<0.05$) yolk color score than those in the control fed with maize based diet. The results demonstrated that in laying hens, up to 40% of maize could be replaced with cassava meal of an appropriate ratio (30% tuber + 10% leaf) as energy source in the diet of laying hen for improving laying performance and egg quality. Moreover, supplementation with cassava leaf (10% and 20%) to tuber was efficient in lowering egg yolk cholesterol contents.

Introduction

Maize is a common feedstuff as a major supplier of energy in monogastric animal diets in Myanmar. It is used about 60% of total diet in the poultry ration, which implies that increasing cost of maize due to low level of production and higher consumption rates by human beings and agro-industries would invariably lead to increased cost of animal feeds (Myanmar Statistical Year Book, 2011). Therefore, the need for replacing maize with non-conventional energy source is considered in order to minimize cost and maximize poultry production (Bamgbose *et al*., 2010). The use of cassava as an alternative to conventional energy feedstuffs like maize could help to reduce feed costs (Anaeto and Adighibe, 2011). However, its use in conventional feed is limited by some factors. The low protein content, essential vitamin and minerals of

cassava tubers have been the major factors limiting its use in poultry diets. Cassava leaf with its high protein content has wide scope as a feed source for poultry and livestock (Rose and Enriquez, 1969). Gomez and Valdivieso (1985) reported that with proper processing techniques, the problem of containing hydrocyanic acid potential (HCNp) can be eliminated. It is therefore proposed that the inclusion of cassava leaf into the cassava tuber might not only increase the level of protein but also improve the mineral and vitamin of the feed might improve the quality of the egg. Report on the substitution of the cassava tuber with various levels of cassava leaf inclusion for maize in poultry feed rations is limited. This study was therefore conducted to evaluate the effect of substituting maize in layer diets with processed cassava tuber and different levels of leaf on laying performance, egg quality parameters and digestibility.

Materials and Methods

1. Experimental diets

Cassava was collected from the field of the University of the Ryukyus, Okinawa. The HCNp contents in leaves and tuber before ensiling were 293.36 mg/kg and 102.44 mg/kg, respectively. All chopped cassava were ensiled by using fermented

juice of epiphytic lactic acid bacteria (FJLB) as a silage additive which were made from napiergrass (*Pennisetum purpureum*). The silos were kept for one month at 29˚C and sun dried before replacement of maize. Sun-dried cassava tuber and leaves were milled separately in a commercial feed milling machine (Sogo's Impact, SOGO, MFG, Co. Ltd., Tokyo, Japan) with 5 mm sieve. The chemical composition of the basal ration was shown in Table 6.1. Four experimental diets were compounded for this study. The basal diet was a typical layer diet containing 2,700 kcal/kg metabolizable energy (ME), 18% crude protein (CP), and equal levels of calcium (3.9%) were calculated to meet or slightly exceed the nutrient requirements recommended by the National Research Council (NRC, 1994). The composition of the experimental diet was shown in Table 6.2. In diet I, II, III and IV, the proportion of maize were replaced with cassava meal of different combination of tuber and leaf: 0% (control), 30% tuber, 30% tuber + 10% leaf, and 30% tuber + 20% leaf, respectively. Other ingredients were added to make a complete feed.

2. Hens and their management

Thirty six white leghorn laying hens of 32-week-old were constituted as the material of this experiment. The laying hens were weighted individually for their allocation to groups comprising of animals with similar live weight. This study was

conducted in four groups including the control group and three trial groups of nine hens each. Each treatment group was replicated three times with 3 hens per replicate in a complete randomize design. The hens were habituated for 2 weeks before the start of feeding experiment. The hens were offered feed twice a day and water were provided on an *ad libitum* basis throughout the experimental period. The standard routine management were carried out which included draining of remaining water, washing of the watering trough and supply of fresh clean cool water, removal of poultry dropping from the remaining feeds in the feeders and addition of fresh feed on daily basis. This study was conducted at the University of the Ryukyus, Okinawa, Japan for a period of 5 weeks from 25 August to 30 September, 2013. Experimental plan and management of fowls was followed the guideline (Prime Minister's Office, 1970). The mean air temperature measured throughout the experimental period was 31˚C.

3. Performance and egg quality

Live weight (kg) measurements were performed at the start of the trial and end of the trial. Feed intake was monitored once a day while egg collection was done twice a day in the morning 9:00-10:00. The number and weight of eggs were recorded daily throughout the experiment. Daily egg production (%) of hens was calculated by the method described by Aderemi *et al*. (2012). Feed conversion ratio (FCR) was

calculated from egg weight and feed consumption. During the treatment, mortality in each group was recorded.

Egg quality measurements were determined once a week using a total of 72 eggs (18 eggs/treatment). The eggs were stored at 4˚C refriengerator for the measurement of egg quality. Parameters measured for egg quality were egg shape index (by the use of a digital caliper), yolk index, Haugh unit, egg shell thickness, egg shell weight and yolk color score. Haugh unit was calculated using the HU formula (Haugh, 1937) based on the height of egg-white determined with a micrometer and egg weight (NABEL-DET-6000, Nabel Co. Ltd., Kyoto. Japan). The detail yolk color pigmentation was measured by using a Color Reader (CR-10, Minolta Co.Ltd., Osaka, Japan). The L^* value indicates the lightness, representing dark to light (0-100). The a^* (redness) value indicates the degree of the red-green color, with a higher positive a^* value indicating more red color. The b^* (yellowness) value indicates the degree of the yellow-blue color, with a higher positive b^* value indicating more yellow color. Egg yolk cholesterol was determined by using a High Performance Liquid Chromatography method (HPLC; Shimadzu Co. Ltd., Kyoto, Japan) described by Bragagnolo and Rodriguez-Amaya (2003).

4. Chemical analysis

Dry matter (DM) content of experimental material was determined by oven

drying at 70˚C to a constant weight. Determinations were made of CP, CF, EE, and ash using the procedures described by AOAC (1985). The gross energy was determined by using Auto-calculating Bomb Calorimeter (CA-4AJ, Shimadzu Co. Ltd., Japan). Cassava tuber and leaf materials were analyzed for the measurement of HCNp contents using acid hydrolysis method (Bradbury *et al*., 1991; Haque and Bradbury, 2002). The concentration of mineral contents in the samples was analyzed by using Inductively Coupled Plasma Atomic Emission spectroscopy (ICPE-9000, Shimadzu Co. Ltd., Japan). Amino acid profile of maize, cassava leaf and tuber used in this experiment were analyzed at Itochu Feed Mills. Co., Ltd, Tokyo, Japan. Pigment composition of experimental diet and eggs were measured in Japan Food Research Laboratories, Tokyo, Japan.

5. Statistical analysis

All data collected were subjected to analysis of varience using one way ANOVA of SPSS 16.0 Software (2007), differences in means was separated using Duncan multiple range test of the same software package.

Results

The chemical composition of the maize and cassava leaf and tuber used in this

experiment were shown in Table 6.1. The HCNp contents were higher in the cassava leaf (14.87 mg/kg) compared with the tuber (4.04 mg/kg). The HCNp contents in the experimental diets (II, III, IV) were ranged from 0.70 to 0.24 mg/kg diet. The results of the statistical analyses of the performance characteristics were shown in Table 6.3. All the groups did not display any statistically significant difference of the performance parameters for feed intake, egg production, egg weight and FCR ($P>0.05$). However, the lowest feed intake value was recorded in the hens fed the control diet. It was determined that at the end of experiment, the hens fed cassava leaf inclusion diet (III and IV) slightly reduced FCR. Average weight gains of layers were similar. No mortality was observed throughout the experimental period.

The egg quality parameters of the hens fed cassava meal diets were presented in Table 6.4. Both the egg shape index and Haugh unit differed significantly among the groups ($P<0.05$). Egg shape index significantly increased ($P<0.05$) as the level of cassava leaf increased. Egg laid by hens fed diets I, II and IV had similar Haugh unit values which were significantly lower ($P<0.05$) than those fed diet III.

Yolk color score was significantly ($P<0.05$) influenced by feeding cassava meal (Table 6.5). Following cassava meal inclusion, yolk color reached a score of 11.78-12.67, which was higher than the control score of 9.22 ($P<0.05$). The visual egg yolk

color score (L^*, a^* and b^*) according to the color reader were also showed in Table 5. Compared with the control diet group, the mean L^*value tended to decrease in the treated diet groups with cassava meal and was significantly ($P<0.05$) lower in diet II. This resulted in a deeper yolk color in treated diet groups with cassava meal. The value of a^* was higher in the cassava meal diet groups and the highest value was observed in diet II. Therefore, the hens fed diets of cassava meal had dark red and yellow color. The pigment composition of experimental materials was showed in Table 6. The contents of β-carotene and lutein were not detected in cassava tuber. However, these pigments were observed in the eggs of cassava dietary treatments (Table 6.7). Vitamin A (retinol) and β-carotene were not detected in the control group eggs.

Egg yolk cholesterol content of hens fed diets I and II were higher ($P<0.05$) than those fed cassava leaf diets (Figure 6.1). However, there was no statistical difference between the content of egg yolk cholesterol at the levels of 10% and 20% cassava leaf substitution in the diets. The lowest value of egg yolk cholesterol content was recorded (9.83 mg/g yolk) with both 10% and 20% cassava leaf diets, which was approximately 24% lower than that of the control diet. Cholesterol content in hens fed control diet was 12.90 mg/g egg yolk.

Table 6.1 Analytical components of maize, cassava leaf and tuber offered for the experiment

Parameters	Maize	Cassava leaf	Cassava tuber
Chemical composition, %			
Dry matter	88.01	92.48	91.67
Crude protein	8.40	16.60	0.90
Crude fiber	1.79	12.70	3.60
Ether extract	4.74	7.00	0.50
Ash	1.42	6.30	3.10
Nitrogen free extract	71.66	55.87	82.80
Gross energy (kcal/kg)	4327	4408	3952
HCNp (mg/kg DM)	ND	14.87	4.04
Mineral composition			
Ca %	0.01	2.00	0.23
Mg %	0.09	0.44	0.12
Zn (ppm)	1.82	56.89	58.98
Fe (ppm)	5.05	144.84	212.05
Amino acids[1], %			
Aspartic acid	0.43	1.67	0.13
Threonine	0.25	0.59	0.07
Serine	0.34	0.74	0.07
Glutamic acid	1.28	1.97	0.20
Proline	0.66	0.70	0.03
Glycine	0.27	0.63	0.07
Alanine	0.50	0.77	0.10
Valine	0.30	0.62	0.07
Cystine	0.17	0.19	0.04
Methionine	0.16	0.20	0.03
Isoleucine	0.19	0.47	0.05
Leucine	0.85	1.06	0.10
Tyrosine	0.14	0.35	0.02
Phenylalanine	0.32	0.73	0.06
Lysine	0.20	0.66	0.09
Histidine	0.21	0.26	0.03
Arginine	0.30	0.63	0.06

[1]Analyzed by Itochu Feed Mills.Co., Ltd, Tokyo, Japan, July 1, 2013; No. R130449A
ND: not detected

Table 6.2 Combination ratio of feed materials in each experimental diet

Ingredients	Diet I	Diet II	Diet III	Diet IV
Substitution rate of corn, %	0	30	40	50
Maize	57.2	40.0	34.3	28.6
Cassava tuber	-	17.2	17.2	17.2
Cassava leaf	-	-	5.7	11.4
Soybean meal	14.2	15.0	14.2	14.2
Wheat bran	1.0	0.5	1.0	1.0
Pork-chicken meal	2.0	2.3	2.0	2.0
Fish meal	2.0	3.0	2.0	2.0
Feather meal	2.0	2.0	2.0	2.0
Ca_3PO_4	0.3	0.3	0.3	0.3
$CaCO_3$	8.8	8.8	8.8	8.8
Fat (oil)	1.1	1.1	1.1	1.1
Gluten meal	3.6	4.0	3.6	3.6
Rapessed meal	4.0	2.0	4.0	4.0
Extra additional formula[¶]	3.7	3.7	3.7	3.7
Total %	100	100	100	100
Calculated analyses				
Crude protein %	18	18	18	18
Lysine %	0.9	0.9	0.9	0.9
Methionine+cystine %	0.5	0.5	0.5	0.5
Calcium %	3.8	3.9	3.9	4.0
Phosphorus (available) %	0.3	0.3	0.3	0.3
ME (kcal/kg)	2780	2756	2720	2708

[¶]Extra additional formula provided percentage of diet: Corn powder: 3%; Salt: 0.25%; Premix: 0.1%; Zeolite, Sea weed, Mugwort and Wood vinegar: 0.3%; Colin chloride (neurotransmitter): 0.01%: Phytase: 0.01%; Pigment: 0.06%; Methionine: 0.002%
Diet I: Control group (no substitutional basal diet); Diet II: 30% tuber; Diet III: 30% tuber + 10% leaf; Diet IV: 30% tuber + 20% leaf

Table 6.3 Performance characteristics of laying hens fed experimental diets

Parameters	Diet I	Diet II	Diet III	Diet IV
Feed intake (g/hen/ day)	95.55±3.93	98.40±3.54	96.48±3.86	101.29±2.46
Egg production (%)	98.33±1.15	97.00±1.00	97.67±0.57	96.33±1.48
Egg weight (g)	57.52±3.74	58.81±2.76	56.72±2.47	59.39±1.94
FCR (kg feed/kg egg)	1.71±0.12	1.71±0.06	1.76±0.11	1.78±0.11
Liveweight gain (kg)	0.06±0.02	0.10±0.05	0.04±0.05	0.08±0.06
Mortality (%)	-	-	-	-

FCR; Feed conversion ratio
Values (mean ± SD) were not significantly different ($P>0.05$) among the treatments.
(Refer to Table 6.2 for the types of Diet)

Table 6.4 Egg quality parameters of laying hens fed experimental diets

Parameters	Diet I	Diet II	Diet III	Diet IV
Egg shape index	73.57±1.42[b]	73.97±1.53[b]	74.43±1.82[ab]	75.92±2.07[a]
Yolk index	0.40±0.01	0.40±0.01	0.40±0.01	0.40±0.00
Yolk weight (g)	15.59±1.66	16.48±1.32	15.38±0.73	16.15±0.69
Haugh unit	89.17±2.83[b]	88.46±2.43[b]	92.45±3.22[a]	88.74±3.33[b]
Egg shell weight (g)	5.41±0.26	5.51±0.41	5.54±0.37	5.72±0.24
Egg shell strength (Kgf)	4.68±0.30	4.45±0.56	4.55±0.25	4.61±0.48
Egg shell thickness (mm)	0.44±0.01	0.44±0.01	0.45±0.01	0.45±0.01

[a-b]Values (mean ± SD) with different superscript on the same row are significantly different ($P<0.05$).
(Refer to Table 6.2 for the types of Diet)

Table 6.5 Effects of experimental diets on yolk color score, lightness, redness and yellowness of egg yolk

Parameters	Diet I	Diet II	Diet III	Diet IV
Yolk color[†]	9.22±0.92[c]	12.67±0.29[a]	11.78±0.27[b]	11.89±0.30[b]
Lightness, L*	59.79±1.12[a]	55.22±0.81[c]	56.37±0.91[b]	56.19±0.93[b]
Redness, a*	7.50±0.50[c]	13.44±0.85[a]	11.67±0.67[b]	11.75±0.81[b]
Yellowness, b*	50.23±1.56[a]	44.39±1.38[c]	46.08±0.97[b]	45.55±1.09[bc]

[a-c]Values (mean ± SD) with different superscript on the same row are significantly different (P<0.05).
[†]Yolk color score obtained from (NABEL-DET-6000, Nabel Co. Ltd., Kyoto. Japan).
(Refer to Table 6.2 for the types of Diet)

Table 6.6 Pigment composition of control diet, cassava leaf and tuber

Parameters	Diet I (control diet)	Cassava leaf	Cassava tuber
β-carotene (mg/100g)	0.08	1.75	ND
Lutein (mg/100g)	0.62	9.10	ND

Analyzed by Japan Food Research Laboratories (Tokyo, Japan, November 18, 2013; No. 13114669001-01 for Diet I, No. 13114669003-01 for cassava leaf and No. 13114669002-01 for cassava tuber)
ND: not detected

Table 6.7 Pigment composition of egg yolk for eggs of dietary treatments

Parameters	Diet I	Diet II	Diet III
Vitamin A (retinol) (μg/100g)	ND	1.00	1.00
β-carotene (μg/100g)	ND	6.00	10.00
Lutein (mg/100g)	1.19	1.01	1.26

Analyzed by Japan Food Research Laboratories (Tokyo, Japan, November 18, 2013; No. 13114669004-01 for Diet I, No. 13114669005-01 for Diet II, and No. 13114669006-01 for Diet III)
ND: not detected
(Refer to Table 6.2 for the types of Diet)

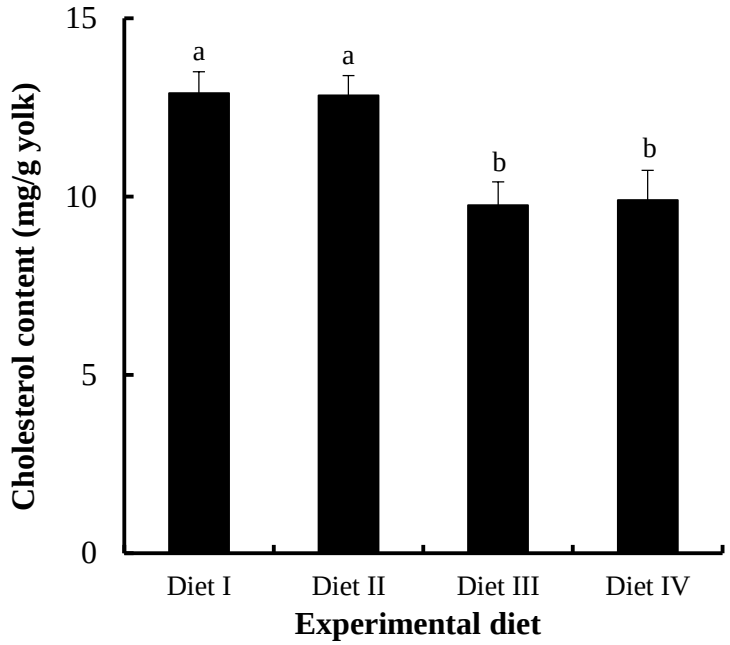


Figure 6.1 Egg yolk cholesterol contents of laying hens fed experimental diets. Values are expressed as the mean ± SD. $^{a-b}$Means with different superscript are significantly different ($P<0.05$). (Refer to Table 6.2 for the types of Diet)

Discussion

The CP content of cassava leaf was higher than that of maize and cassava tuber (Table 6.1). The HCNp content was higher in cassava leaf than in tuber, which agreed the results of Onwueme and Charles (1994). The amino acid composition of maize, cassava leaf and tuber used in this experiment are typical to those reported by Yeoh and Chew (1976), who stated that cassava leaf was not only rich in proteins but also in the essential amino acids. Muller *et al*. (1974) also reported that the amino acid profile in cassava leaf was rich in lysine compared with napiergrass, Gatton panic (*Panicum maximum*) and soybean meal, while the protein in maize was poor in this amino acid.

The lack of significant difference in feed intake was probably due to small difference in ME values of the diet groups, because, the difference between the diets with the highest and the lowest ME of the control and diet IV was 72 kcal/kg. The highest feed intake value was also observed in diet IV (101.29 g/hen/day) while the lowest was in the control diet (95.55 g/hen/day). The similarity was observed in feed intake in the other's report who stated that layers received high fiber from the low energy cassava meal based diet had higher feed intake, because low energy diets stimulate feed intake (Osei *et al*., 1990). Egg production revealed that there was

similarity among layers on diet I to IV. This is an indication that the inclusion of cassava meal in layer diets adequately maintained sustaining efficiency of egg production. However, the result showed that the cassava leaf 20% inclusion slightly decreased egg production compared with 10% leaf inclusion. This may be due to the increase in the amount of dietary fiber as the cassava leaf increased in the diet. Fiber had been reported to form complexes with other nutrients that preventing their breakdown and utilization thus egg production decreased (Aderemi *et al*., 2012). This observation indicated that layers could tolerate cassava meal 40% replacement including tuber 30% and leaf 10% for maize without adverse effect on egg production.

The efficiency of FCR was reduced by the increased levels of cassava leaf inclusion to the experimental diet, a finding that corroborates the earlier observation of Osei *et al*. (1990). The reduced efficiency of FCR may be attributed to the high fiber and low energy content of cassava meal (Ijaiya *et al*., 2002). Enriquez and Ross (1967) have reported HCNp toxicity for poor FCR, but it is unlikely that HCNp was responsible in this experiment as extremely low levels were involved (Table 1), which are in agreement with Osei *et al*. (1990). Hens in this study gained some weight at the end of the experiment. This is an indication that the diets were adequate in nutrient composition to sustain egg production and growth. The results obtained however did

not agree the finding of Stevenson (1984) who reported that a rate up to 50% cassava in the diet impaired the growth performance of poultry. It was also reported that HCNp causes a reduction in growth rate by inhibiting intra-thyroidal uptake of iodine, causing increase in secretion of thyroid stimulating hormone and thereby causing a reduction in thyroxin level which is necessary for growth (Tewe, 1994). However, Tewe (1991) also reported that the HCNp contained in cassava should not be a problem as sun drying is known to reduce the level of these compounds to the point where they have no negative effect on the animal. The weight increase observed in this study could be due to the fact that the hens were still growing physiologically and the similar results were observed in Oladunjoye *et al*. (2010). The absence of mortality among all the experimental hens showed that cassava meal may have been innocuously reduced of its HCNp content during ensiling and sun drying, which agrees previous finding of Tewe (1991). The hens were apparently healthy during the experiment.

There was a significant increase in egg shape index as 20% cassava leaf was incorporated in layer diet which was larger than that of standard egg of chicken 74 (Powrie, 1977). It is suggested that the increase in egg weight was due to decrease in egg numbers since egg weight is inversely related to the number of eggs produced

(Osei *et al*., 1990). Moreover, Hammershoj and Steenfeldt (2005) observed that decrease in feed intake may lead to inadequate supply of essential amino acids and, especially if the methionine intake is below requirement, the egg weight may drop. In this study, the highest value of Haugh unit was recorded with 10% leaf inclusion and it declined again in 20% leaf inclusion. Oladunjoye *et al*. (2010) reported that the decreasing Haugh unit in cassava treating diets could be due to HCNp in cassava leaf which probably reached threshold level in these diets. Moreover, Roberts (2004) stated that many factors affected Haugh unit such as storage time and temperature, hen age, strain of bird, nutrition (dietary protein and amino acid content e.g. lysine, methionine, feed enzymes, grain type/protein source), disease (IB), supplements (ascorbic acid, vitamin E) and artificial exposure to ammonia. Therefore, further research may focus to identify the factors effecting Haugh unit by the inclusion of cassava leaf in layer diet. Yolk weight and yolk index were not influenced by implication the inclusion of cassava meal in layer diets. Egg shell weight, egg shell thickness and egg shell strength were not significantly affected by the dietary treatments. No changes in egg shell quality were observed in this experiment because the experimental diets were adequate in calcium.

The higher yolk color scores were observed in the eggs of the laying hens fed

cassava meal diets. Rose (2005) stated that laying hens cannot synthesize egg yolk pigments and egg yolk color closely depends on the fat-soluable pigments mainly xanthophyll, lutein, zeaxanthine and β-cryptoxanthine in the diets fed. These pigments provide different colors, from light yellow to dark red (Surai *et al*., 2001). Because the hens in all groups were fed the same basal diet, except for the replacement of dietary cassava tuber and leaf in group II, III and IV: The higher color intensity of yolk in the cassava meal group might have been induced by the cassava materials. The value of β-carotene in cassava leaf used in this study clearly showed that cassava leaf has high carotene content which enriches the yolk color of egg. In this experiment, β-carotene and lutein were not detected in tuber, that is in line with Oladunjoye *et al*. (2010), who had earlier reported that the reduction in egg yolk color score of birds fed cassava peel-based diets could be due to less pigmentation of cassava peel. However, the higher value of redness a^* color score was observed in diet II (only tuber 30%) treatment. It might be the different percentage of corn gluten meal in the experimental diet (Table 6.2), because Bailey and Chen (1989) reported that corn gluten meal is one of the primary sources of xanthophylls.

Cholesterol concentration of egg yolk in the control group was similar to the values reported by Bragagnolo and Rodriguez-Amaya (2003). Significantly low yolk

cholesterol value was observed in hens ($P<0.05$) with 10% and 20% leaf inclusion, which attributed higher fiber content of the diets. This supports the hypothesis that increased dietary fiber often result in reduction in availability of cholesterol for incorporation into lipoproteins (Story and Furumoto, 1990). Moreover, the presence of HCNp in cassava leaf can exert hypocholesteronic influence as glycosides have ability to interfere with the intestinal absorption of the dietary cholesterol and lipid (Brown *et al*., 1999).

Conclusion

This study indicated that cassava leaf should be added along with tuber in replacement of maize in layer diet. Because cassava leaf is an appreciable source of carotenoids especially lutein and it could be used for adequate carotenoids supply for acceptable egg yolk color. In addition, high level of Vitamin A, β-carotene, and lutein and low level of cholesterol in eggs resulted from laying hens fed a diet with cassava tuber and leaf in replacement of maize. Among four substitution ratios of corn, 40% cassava substitution (30% tuber + 10% leaf) was the best in terms of egg production and Haugh unit score, as well as keeping good health condition of hens.

CHAPTER 7

Effect of whole cassava meal as substitutes for maize in the diets on the performance characteristics and egg quality of laying hens

Abstract

The effects of feeding diets containing cassava meal (tuber and leaf) were investigated on feed intake, feed conversion ratio, weight gain, egg performance, egg quality and digestibility of layer during 4 weeks of feeding experiment. Thirty-six white leghorn layer were allocated into 4 groups of nine hens each. Diet I, contained no cassava meal but maize 57% of the diet and served as control. In diet II, III and IV, the proportion of maize was replaced with the cassava meal at the levels of 50% (40% tuber + 10% leaf), 75% (65% tuber + 10% leaf), and 100% (90% tuber + 10% leaf), respectively. The cassava meal diets tended to reduce dry matter digestibility. The birds were able to tolerate up to 75% of cassava meal (65% tuber + 10% leaf) after which egg production declined. Cassava meal diet did not have significant ($P>0.05$) dietary effects on the egg quality parameters. However, the trend of yolk color score decreased to the increase of the substitution level of cassava meal. The results demonstrated that maize (57%) in the commercial formula feed could be replaced

with cassava meal up to 75% without any adverse effects on the laying performance and egg quality.

Introduction

Cereal grains, particularly maize, normally constitute a major proportion of poultry feed as the energy source. Maize is also the main source of energy in most farm animal feeds. However, the situation is different in the developing countries where cereals, legumes and some other animal feed components constitute staple foods for man (Eruvbetine *et al*., 2003; Onyimonyi and Ugwu, 2007). In the coming years, poultry producers will definitely have to look beyond maize and other cereal grains because of their low availability and inability to keep pace with ever-increasing poultry production (Chauynarong et al., 2009).

Oruwari *et al*. (2003) also stated that a complete substitute of maize was enabled by the addition of brewer's yeast to cassava tuber, and increasing protein content in ratioanl feed for broiler. Therefore, cassava tuber meal might be completely replace maize in poultry diets with proper protein balance. However, the content of hydrocyanic acid potential (HCNp) of the leaf is six times higher than that of the tuber (Reeds *et al*., 1982). Different physical processing methods have been tried for

cassava, including sun drying, boiling, mashing and pelleting, to improve the nutritive value and reduce HCNp content. Gomez *et al*. (1984) reported that more than 86% of HCNp present in cassava was lost probably due to the evaporation of free cyanide on sun drying at about 28˚C. Substantial efforts have been made in the past to replace cereals with cassava in poultry feeding but the response, in terms of productive performance of laying hens fed cassava products, has been widely variable. Moreover the information, regarding its utilization by substitution of the various levels of cassava tuber incorporated with 10% leaf for maize in poultry feed rations, is limited. It would seem to be more desirable and advantageous if cassava could be used to completely replace maize in layer feeds. This study was therefore conducted to evaluate the effects of complete replacement of maize with cassava meal on the performance and egg quality of laying hens.

Materials and Methods

1. Experimental diets

Cassava was collected from the field of the University of the Ryukyus, Okinawa. The HCNp contents in leaves and tuber before sun-drying were 103.25 mg/kg and 82.43 mg/kg, respectively. Sun-dried cassava tuber and leaves were milled separately

in a commercial feed milling machine (Sogo's Impact, SOGO, MFG, Co. Ltd., Tokyo, Japan) with 5 mm sieve. All diets were formulated to meet or exceed the NRC (1994) requirements for laying hens and provided in mash form. The composition of the experimental diet is shown in Table 7.2. In diet I, II, III and IV, the proportion of maize were replaced with the cassava meal at the levels of 0% (control), 50% (40% tuber + 10% leaf), 75% (65% tuber + 10% leaf), and 100% (90% tuber + 10% leaf), respectively. Calcium soap fatty acid (Bypass mate L, YUKA SANGYO, Co. Ltd., Tokyo, Japan) was added into diet III and IV to meet the energy requirements recommended by NRC (1994) at the level of 2% and 3%, respectively. The chemical composition of the basal ration was shown in Table 7.1.

2. Hens and their management

The 52 week-old thirty six white leghorn laying hens constituted the material of this experiment. The laying hens were weighted individually for their allocation to groups comprising of animals with similar live weight. This study was conducted in four groups including the control group and three trial groups of nine hens each. Each treatment group was replicated three times with 3 hens per replicate in a complete randomize design. Before the beginning of the experiment, hens were provided with a basal diet for a 14 days adjustment period. The experimental diets and water were

provided for *ad libitum* intake. The standard routine management were carried out which include draining of remaining water, washing of the watering trough and supply of fresh clean cool water. Removal of poultry dropping from the remaining feeds in the feeders and addition of fresh feed on daily basis. This study was conducted at the University of the Ryukyus, Okinawa, Japan for a period of 4 weeks from 25 January to 8 March, 2014. Experimental plan and management of fowls was followed the guideline (Prime Minister's Office, 1970). The mean air temperature measured throughout the experimental period was 21˚C.

3. Performance, egg quality and digestibility

Laying performance and egg quality analysis were carried out by the use of methods described in Chapter 6. Dry matter (DM) digestibility trial of experimental diets was performed by the method described by Takemasa (2001). The digestibility and fecal output trial was done for 10 days at the end of this experiment. Each treatment group was replicated three times with 3 hens per replicate and conducted with the same experimental diets. The birds were placed into individual battery cages and fecal drops were collected in the same time throughout the digestibility trial period. The excrement samples were oven-dried at 70˚C for 48 hours and then were ground for chemical analyses.

4. Chemical analysis

Dry matter (DM) content of experimental material was determined by oven drying at 70˚C to a constant weight. Determinations were made of CP, CF, EE, and ash using the procedures described by AOAC (1985). The gross energy was determined by using Auto-calculating Bomb Calorimeter (CA-4AJ, Shimadzu Co. Ltd., Japan). Cassava tuber and leaf materials were analyzed for the measurement of HCNp contents using acid hydrolysis method (Bradbury *et al*., 1991; Haque and Bradbury, 2002).

5. Statistical analysis

All data collected were subjected to analysis of varience using one way ANOVA of SPSS 16.0 Software (2007), differences in means was separated using Duncan multiple range test of the same software package.

Results

The results of the chemical composition and gross energy (GE) of maize and cassava before utilizing as a layer diet were presented in Table 7.1. The proximate composition, metabolizable energy (ME) values which was calculated by the method described by Fisher and Boorman (1986) and HCNp content of experimental diets were also shown in Table 7.3. The crude fiber and fat (ether extract) contents were

significantly increased (P<0.05) with the increased substitution of cassava meal. All the groups did not display any statistically difference (P>0.05) of the DM digestibility of all experimental diets (Table 7.4). However, it tended to decrease in cassava meal treated groups compared with the control diet groups.

The results of the statistical analyses of the performance characteristics of laying hens fed experimental diets were summarized in Table 7.5. Dietary treatments had no significant (P>0.05) effects on body weight, feed intake and egg weight. However, the laying hens fed cassava diet (III and IV) slightly decreased the final body weights compared with the control group. This is an indication that the cassava meal replacement for maize up to 50% adequately maintained body weight. The egg production values recorded in control hens and those laying hens fed cassava meal diet were 83.71%, 90.12%, 87.24% and 72.43%, respectively. According to this results, the egg production of the laying hens fed diet IV groups was significantly lower (P<0.05) than the control and other groups. Laying hens in diet II and III treatments had significantly lower (P<0.05) FCR values than those in control and diet IV treatments during the experiment. No mortality was observed throughout the experimental period.

Egg quality characteristics were shown no significant difference (P>0.05) among the treatments (Table 7.6). In relation to egg internal quality as measured with Haugh units, no effects (P>0.05) of different levels of cassava meal diet were

observed. All the groups did not display any statistically significant difference for egg yolk cholesterol content. However, the lowest cholesterol content was recorded (14.69 mg/g yolk) in the hens fed the control diet. It was determined that the laying hens fed cassava meal diet III and IV slightly increased cholesterol content to the levels of 15.25 mg/g yolk and 15.90 mg/g yolk, respectively.

Yolk color score was significantly ($P<0.05$) affected by feeding cassava meal (Table 7.7). Yolk color score decreased in proportion to incremental cassava meal level in the diets with diet II, III and IV having the score of 10, 10, and 9. In addition, the visual egg yolk color score (L^*, a^* and b^*) according to the color reader were also shown in Table 7.7. A diet containing cassava meal significantly affected to the yolk color that mainly decreased the redness a^* value from 14.57 for the control to 11.42 for the diet IV, but without affecting the L^* and b^* values. The yellowness b^* values were similar ($P>0.05$) between dietary treatments ranging from 53.59 to 55.77.

Table 7.1 Analytical components of maize, cassava leaf and tuber offered for the experiment

Parameters	Maize	Cassava leaf	Cassava tuber
Chemical composition, %			
Dry matter	88.01	92.24	91.86
Crude protein	8.40	24.00	5.00
Crude fiber	1.79	11.80	4.02
Ether extract	4.74	6.20	1.10
Ash	1.42	7.50	4.90
Nitrogen free extract	71.66	42.74	76.84
Gross energy (kcal/kg)	4326	4616	3550
HCNp (mg/kg DM)	ND	18.24	16.43

ND: not detected

Table 7.2 Combination ratio of feed materials in each experimental diet

Ingredients	Diet I	Diet II	Diet III	Diet IV
Substitution rate of corn, %	0	50	75	100
Maize	57.2	28.6	14.3	-
Cassava tuber	-	22.9	37.2	51.5
Cassava leaf	-	5.7	5.7	5.7
Soybean meal	14.2	14.2	14.2	14.2
Wheat bran	1.0	1.0	1.0	1.0
Pork-chicken meal	2.0	2.0	2.0	2.0
Fish meal	2.0	2.0	2.0	2.0
Feather meal	2.0	2.0	2.0	2.0
Ca_3PO_4	0.3	0.3	0.3	0.3
$CaCO_3$	8.8	7.8	7.8	7.8
Calcium soap fatty acid	-	1.0	1.0	1.0
Fat (oil)	1.1	1.1	1.1	1.1
Gluten meal	3.6	4.0	4.0	4.0
Rapessed meal	4.0	3.6	3.6	3.6
Extra additional formula[¶]	3.7	3.7	3.7	3.7
Total %	100	100	100	100
Calculated analyses				
Crude protein, %	18	18	18	18
Lysine, %	0.9	0.9	0.8	0.8
Methionine+cystine, %	0.5	0.5	0.5	0.5
Calcium, %	3.8	3.8	3.8	3.8
Phosphorus (available), %	0.3	0.3	0.3	0.4
ME (kcal/kg)	2780	2724	2788	2777

[¶]Extra additional formula provided percentage of diet: Corn powder: 3%; Salt: 0.25%; Premix: 0.1%; Zeolite, Sea weed, Mugwort and Wood vinegar: 0.3%; Colin chloride (neurotransmitter): 0.01%: Phytase: 0.01%; Pigment: 0.06%; Methionine: 0.002%
Diet I: Control group (no substitutional basal diet); Diet II: (substitution rate of corn) 40% tuber + 10% leaf; Diet III: 65% tuber + 10% leaf; Diet IV: 90% tuber + 10% leaf

Table 7.3 Chemical composition of experimental diets

Parameters	Diet I	Diet II	Diet III	Diet IV
Chemical composition, %				
Dry matter	91.36±0.01	92.23±0.04	92.67±0.11	93.11±0.76
Crude protein	20.00±0.02^{b}	20.70±0.32^{a}	17.62±0.32^{d}	19.05±0.38^{c}
Crude fiber	1.69±0.01^{c}	2.92±0.06^{b}	3.05±0.20^{ab}	3.16±0.11^{a}
Ether extract	3.02±0.01^{c}	2.89±0.02^{c}	3.94±0.14^{b}	5.14±0.30^{a}
Ash	13.03±0.18^{d}	13.69±0.01^{c}	14.50±0.11^{b}	16.81±0.55^{a}
Nitrogen free extract	53.62±0.20^{a}	52.03±0.32^{b}	53.57±0.60^{a}	48.95±0.40^{c}
ME‡ (kcal/kg)	2888.10±7.64	2847.50±2.98	2872.30±3.86	2858.90±12.76
HCNp (mg/kg DM)	ND	4.80±0.01	7.15±0.02	9.50±0.02

$^{a-d}$Values (mean ± SD) with different superscript on the same row are significantly different ($P<0.05$).
‡Metabolizable energy value was calculated using the method 37 x %CP + 81 x %Fat + 35.5 x %NFE for poultry (Fisher and Boorman, 1986)
ND: not detected
Refer to Table 7.2 for the types of Diet

Table 7.4 Digestibility and metabolizable energy of experimental diets

Parameters	Diet I	Diet II	Diet III	Diet IV
Feed				
DM, %	91.36±0.01	92.23±0.04	92.67±0.10	93.11±0.72
Gross energy (kcal/kg)	4026.20±17.84[a]	4001.90±13.21[b]	3912.70±10.13[c]	3993.50±17.71[b]
Feces				
DM, %	24.93±2.67[a]	17.67±1.55[b]	19.27±0.81[b]	22.93±1.03[a]
Gross energy (kcal/kg)	3266.00±10.27[a]	3099.30±23.59[b]	3156.80±37.26[b]	3305.50±7.52[a]
DM digestibility, %	75.13±2.88	73.79±2.94	71.36±2.62	70.09±2.67
Gross energy (kcal/hen)	428.52±7.48	439.90±2.71	427.94±5.74	429.66±9.94

[a-d]Values (mean ± SD) with different superscript on the same row are significantly different (P<0.05).
Refer to Table 7.2 for the types of Diet

Table 7.5 Performance characteristics of laying hens fed experimental diets

Parameters	Diet I	Diet II	Diet III	Diet IV
Initial weight (kg/hen)	1.73±0.24	1.78±0.23	1.73±0.19	1.73±0.26
Final weight (kg/hen)	1.75±0.26	1.78±0.21	1.72±0.22	1.72±0.29
Feed intake (g/hen/ day)	106.42±2.44	110.16±0.68	109.35±1.46	107.37±2.87
Egg production (%)	83.71±3.41[a]	90.12±3.26[a]	87.24±2.01[a]	72.43±3.10[b]
Egg weight (g)	63.06±3.69	64.88±2.02	65.39±3.21	65.13±2.54
FCR (kg feed/kg egg)	2.09±0.20[a]	1.78±0.13[b]	1.84±0.12[b]	2.11±0.16[a]
Mortality (%)	-	-	-	-

FCR; Feed conversion ratio

[a-b]Values (mean ± SD) with different superscript on the same row are significantly different (P<0.05).

Refer to Table 7.2 for the types of Diet

Table 7.6 Egg quality parameters of laying hens fed experimental diets

Parameters	Diet I	Diet II	Diet III	Diet IV
Egg shape index	72.97±1.33	72.74±1.13	73.34±2.14	73.97±1.45
Yolk index	0.32±0.04	0.30±0.00	0.32±0.04	0.33±0.05
Yolk weight (g)	18.09±1.22	18.66±1.06	17.84±0.86	18.34±0.91
Haugh unit	86.12±2.35	86.51±2.71	86.40±2.74	86.49±1.96
Egg shell weight (g)	6.23±0.45	6.29±0.35	6.46±0.36	6.29±0.17
Egg shell strength (Kgf)	4.74±0.63	4.93±0.37	4.98±0.54	4.61±0.36
Egg shell thickness (mm)	0.46±0.01	0.46±0.01	0.45±0.01	0.46±0.01
Yolk cholesterol (mg/g yolk)	14.69±1.45	14.85±1.14	15.25±1.29	15.90±2.75

Values (mean ± SD) were not significantly different (P>0.05) among the treatments.
Refer to Table 7.2 for the types of Diet

Table 7.7 Effects of experimental diets on yolk color score, lightness, redness and yellowness of egg yolk

Parameters	Diet I	Diet II	Diet III	Diet IV
Yolk color[†]	11±0.50[a]	10±0.60[b]	10±0.70[b]	9±0.52[b]
Lightness, L*	59.57±1.31	59.42±1.09	59.65±0.61	60.73±1.19
Redness, a*	14.57±0.36[a]	13.24±0.63[b]	12.43±0.49[c]	11.42±0.29[d]
Yellowness, b*	55.60±2.40	55.77±2.08	53.59±1.56	54.37±1.45

[a-d]Values (mean ± SD) with different superscript on the same row are significantly different ($P<0.05$).
[†]Yolk color score obtained from (NABEL-DET-6000, Nabel Co. Ltd., Kyoto, Japan).
Refer to Table 7.2 for the types of Diet

Discussion

The calculated nutritional data presented in Table 7.2 were based on earlier analytical data of maize, cassava leaf and tuber obtained from Table 7.1. The analyzed ME values in the experimental diets shown in Table 7.3 were observed similarly with the control diet but lower ME values when compared with 3870 kcal/kg ME of unpeeled cassava tuber meal (Tion and Adeka, 2000). The differences in nutrient composition of cassava relative to previous studies might be due to variety differences, differences in soil conditions and rainfall distribution (Osei and Twumasi, 1989). An addition of calcium soap fatty acid to laying hen diets affected the crude fat percentage increased to 3.94% and 5.14% in diet III and IV, respectively. The lower HCNp content in cassava diet treatments could be due to the effect of sun drying before utilizing as a layer diet. Based on the HCNp contents of cassava leaf and tuber, the HCNp contents were calculated to be 4.80 mg/kg, 7.15 mg/kg and 9.50 mg/kg in the diet II, III and IV treatments, respectively. The HCNp contents in the cassava meal diet treatments in this study were lower than the values reported by Oladunjoye *et al*. (2010). Panigrahi (1996) described that an excess of HCNp content at 100 mg/kg diet appears to adversely affect broiler performance, and laying hens may be affected by levels as low as 25 mg/kg.

The decline in DM digestibility in proportion to increasing cassava meal in the present experiment might probably be due to the presence of dietary fiber in diet III and IV (Table 7.4) because the cassava tubers used in this study were not peeled out. Adeyemo *et al*. (2013) stated that cassava peel has high fiber content that makes it indigestible to monogastrics when included in their feed. Therefore, the rate of nutrient release may be slow hence the absorption rate is also slow. This will not allow the laying hens to get the required essential nutrients for growth (Anthony, 2009).

The changes of body weight were not observed in the laying hen fed diet II and this finding was consistent with Stevenson and Jackson (1983) who found that weight was not affected by a diet containing 50% cassava tuber meal. The decline in body weight in the present experiment may, however, have been due to the presence of high fiber content of cassava diet treatments (III and IV). Weiss and Scott (1979) reported that fiber has been known to depress intake and increase bulkiness and consequently cause growth depression.

The similarity that was observed in feed intake of laying hens fed maize based diet and cassava meal diet is in agreement with Oladunjoye *et al*. (2010), but contrary to Anaeto and Adighibe (2011), who found that decreased feed intake in layers fed

cassava root meal. It would be expected that the addition of calcium soap fatty acid to the experimental diets adjusted ME values and improved the feed intake.

Hen day production revealed that there was similarity among layers fed diet I, II and III. This implied that cassava meal could replace maize up to 75% in layers without adverse effect on egg production. On the other hand, the result showed that the egg production decreased in the case of increasing cassava meal replacement over 75%. From this result, feed intake increased in laying hens fed diet IV but not enough to improve egg production. This can be attributed to residual HCNp content which probably reached an intolerable level in these diets (Oladunjoye *et al*., 2010). Moreover, fiber had been reported to form complexes with other nutrients thereby preventing their breakdown and utilization, and thus the egg production decreased (Aderemi *et al*., 2012). Results of the present study are in harmony with the reports of Aina and Fanimo (1997) who observed a decrease in egg production with cassava meal wholly replaced maize, where feed intake was not affected.

Considering the overall mean, laying hens fed cassava meal diets laid slightly heavier eggs than the control. The positive relationship between increased dietary supplemental fat and egg weight are also well documented (Grobas *et al*., 2001). The improved egg weight of laying hens fed diets III and IV would be expected as a result

of addition of dietary calcium soap fatty acid to the experimental diets. The FCR showed that the improvement with the use of cassava meal up to 75% level due to considerable higher egg production in diet II and III than diet IV treatments. The efficiency of FCR was depressed by the increased levels cassava meal to the experimental diets (diet IV), a finding that corroborates the earlier observation of Osei *et al*. (1990). Ijaiya *et al*. (2002) observed that cassava peel meal reduced FCR due to a high dietary crude fiber level. The significantly highest FCR recorded also for layers fed control diet may have resulted from slightly lower egg production and decreased egg weight compared with the treatments II and III.

In this experiment, egg shape index values were ranged from 72.74 to 73.97 for eggs from the laying hen fed diet I to diet IV, with the medium average of 73.26. According to Romanoff and Romanoff (1949), the standard eggs from hens had a shape index of 74 with blunt and pointed ends. The egg shape index, which is ranging from 70 to 77, can be estimated as an optional value. Other higher and lower quantities would point out more rounded, more elliptic or more elongated eggs (Nikolova and Kocevski, 2006). Yolk weight and Haugh unit did not have a particular statistical trend by a replacement with the cassava meal for the maize in layer diets and this is in agreement with the reports of Aderemi *et al*. (2012). The Haugh unit

value recorded for all treatments in this experiment were within the range of freshly-laid eggs (Essien, 1990). The non significance of egg shell qualities (egg shell weight, egg shell strength, egg shell thickness) implied all the dietary treatments were adequate in calcium which was similar to a finding that cassava based diet did not interfere with calcium metabolism in the laying hen (Aderemi *et al*., 2012). Cholesterol concentration of egg yolk in the control group was slightly higher than the values reported by Bragagnolo and Rodriguez-Amaya (2003) but lower than the values that reported by EI Bagir *et al*. (2006). Natural variation between samples has also been reported, the cholesterol level in eggs varying with species, breed, hen's age, egg and yolk weight, and diet (Jiang and Sim, 1991). Moreover, the fatty acid composition of egg lipids in laying hens can be influenced predictably by the fatty acid composition of the diet (Beynen, 2004). Therefore, the slight increase in the egg yolk cholesterol might be due to the addition of calcium soap fatty acid to the cassava meal diets of III and IV treatments.

A decrease in a^* value of egg yolk was one of the reasons for causing yolk color pale in the egg of laying hens fed cassava meal. Hens are not able to synthesise color pigments, but have the ability to transport about 20-60% of pigments to the yolk from ingested feed (Bartov and Bornstein, 1980). Adewusi and Bradbury (1993) reported that white-color cassava tuber may contain only small amounts of β-carotene. We

used white color tuber in the present experiment and β-carotene and lutein were not detected in these cassava tuber. In this experiment, 10% of cassava leaves were incorporated with tuber in respective cassava meal diet treatments (II, III and IV). Buitrago (2009) also established that cassava leaf has high carotene content which enriches the yolk color of the egg. However, the proportion of substituted cassava tuber for maize was markedly high in this experiment, which corresponds to pale yolk color score of the eggs. This finding was consistent with Oladunjoye *et al*. (2010), who had earlier reported that the reduction in yolk color score of laying hens fed cassava peel-based diets could be due to less pigmentation of cassava.

Conclusion

It is concluded that the acceptance of cassava-based ration as measured by feed intake shows that cassava could be used in poultry industry, assuming reducing HCNp contents beforehand. Moreover, the utilization of cassava meal up to 75% (65% tuber + 10% leaf) improved egg production and feed conversion efficiency without adverse effects of the health of laying hens and egg quality. Therefore, these results are certainly interesting and have great potential value for the layer industry where there is scarcity of maize.

CHAPTER 8

General Discussion

1. HCNp reduction by processing methods

It is confirmed by the experiment that the processing techniques such as soaking or wilting to raw cassava tuber must make reducing the HCNp contents, irrespective of their initial contents. This study revealed that soaking cassava products have higher HCNp reduction levels (62-66%) than wilting (51-52%). After 48 hours of wilting period in preliminary experiment, fungus growth started on the surface of tuber. The soaking process is also faster and simpler than that of silage making and most of the farmer used in Myanmar. However, the disadvantage of soaking method is a blackish colouration was observed on the tubers after 48 hours of soaking period. This could be the reduction of iron (III) oxide to iron (II) oxide which is more readily utilized by bacteria.

Ensiling was highly effective in substantially reducing the HCNp contents of cassava leaves and tuber. The HCNp contents diminished gradually during the first 7 days of storage and then decreased at a rapid rate until 14 days of storage period. From the results of the present study, fermentation quality of the cassava leaves and tuber silage fermented with FJLB additives meets the criteria of well-preserved silage. The

additive used to prepare the silage in our study was leucaena and napiergrass, which are year round products made by rural farmers even in Myanmar, and therefore is readily available and inexpensive for preserving cassava leaves and tuber. From the practical relevance of this research, it can be concluded that both additives improved silage quality and reduced HCNp contents which were observed in Red and White cassava varieties varying in an initial HCNp content of fresh one. Therefore, ensiling cassava leaves and tuber by using FJLB additives are a good preservation method especially when harvest coincides with the wet season.

The present data suggested that it is possible to reduce the HCNp contents from the cassava materials by using simple processing techniques. While ensiling reduced HCNp contents in both leaves and tuber to moderately toxic levels (50-100 mg/kg), the processing techniques were efficient with regard to HCNp removal for low HCNp level of cassava variety. However, it should be noted that the values for initial HCNp contents in cassava used in this study were relatively low compared with other studies. All the cassava leaves samples in finalized products were not below the limit (<25 mg/kg HCNp) as safe utilization for poultry by Panigrahi (1996). Furthermore, the wide range in concentration of HCNp value in over 5000 known phenotypically distinctive cassava cultivars (Best and Hargrove, 1993) is an indication that genetic detoxification

is feasible. This indicated that further study needs to be done on the detoxification level of the cassava as to reduce or eliminate the cyanic poisoning to a tolerable level.

2. HCNp reduction by management strategies

Studied were therefore initiated on the improvement of the cassava products with low HCNp contents for utilization such as poultry feed. The initial HCNp contents were vary in both Experiments that were stated in Chapter 4 and 5. The samples presented in Table 4.2 were from 12 months old plant harvested in June, 2012 while those in Table 5.2 and 5.3 were from 7 months old plant harvested in October, 2013. The difference of initial HCNp content between the plants used in each experiment were probably due to harvesting age, environmental temperatures, planting space and subsequent harvestings. Protein content is one of the most important parameter evaluating the nutritional value of forage crop. This study indicated that the high potential of cassava leaves as an unconventional protein resource for both monogastric and ruminant livestock.

In our study, some cassava plots especially control plot had unacceptable values of HCNp over 100 mg/kg meanwhile average values were 62 mg/kg of HCNp content in fertilized plots. Therefore, it was quite obvious that the two application levels, F4 and F5 (50 kg N, 100-250 kg K_2O) had the advantages over all treatments as evidenced in their lower HCNp contents and higher tuber yield per plant than control treatments.

Moreover, our studies revealed that the negative correlation between the HCNp content and crop yield. Although potassium is observed important in reducing the HCNp content of cassava, other locally available and cheap source of potassium such as wood ash can alternatively be used by the mainly subsistent farmers who usually cultivate the crop.

Based on this result from Chapter 5, it can be concluded that different initial harvesting intervals of cassava foliage affected to chemical composition of cassava. Early initial harvesting of cassava foliage showed that CP content tended to be higher and fibrous fractions lower. The control treatment gave a somewhat lower foliage yield but slightly lower HCNp content compared with other treatments. As the CP content of leaves on DM basis had 2 to 4 times higher than stem plus petiole. Therefore, it is important to separate leaves from stem plus petiole when feeding cassava foliage, especially to poultry. The root cortex had 6 times higher CF content than the parenchyma. The digestibility and utilization of carbohydrates in poultry is highly dependent on the proportion of starch and dietary fiber in the diet.

From the results of two years field experiments (Chapter 4 and 5), it may be concluded that management strategies in combination with subsequent harvesting might be produced good quality forage with lower HCNp level. It is evident that fertilization

or harvesting management can result in significant HCNp detoxification and improved foliage quality, respectively. Hence, the HCNp content in the final products can be further reduced for safe utilization when combined with ensiling HCNp reducing method.

3. Feeding values of cassava diets

Several have reported that, due to its bulkiness and high fiber content, the maximum level of inclusion of cassava leaf in diets is between 10-20% for monogastrics animals (Eruvbetine *et al*., 2003). In this present experiment, the optimal incorporation rate was observed as 10% cassava leaf. Cassava leaf supplementation is a simple and convenient strategy to transfer carotenoids to the egg adding value to an already a valuable nutritional and functional food. Egg rich in vitamin A, and β-carotene resulted from laying hens fed a cassava diet incorporated with 10% dry cassava leaf.

Dietary inclusion of cassava meal did not affect the health of the layers during the experiment in both feeding trials. With regards to egg quality, these trials showed that the fresh eggs produced by each of the four treatment diets fell within the range of normal egg sizes; the yolk index values of the eggs were within the reported range of 0.30-0.50 for fresh eggs; and their shell thickness values of >0.33 mm, indicating that the eggs will not crack easily during handling/transportation. It might be necessary to

maintain total dietary levels of xanthophyll pigment by appropriate diet formulation when using high levels of cassava tuber meal in layers feed to avoid having a decline in the intensity of yolk pigmentation. In the main inclusion of 75% (65% tuber + 10% leaf) cassava meal was found economical and productive as it was able to support body maintenance and egg production of the hen at a level which was comparable to those on control.

These cassava based diets were cheaper to produce than commercial diet in many countries of the region, e.g Myanmar and this reflected on the lower cost of feed consumed per kg egg produced. These attractive economic gains obtained from replacing all of the maize with cassava is certainly interesting and has great potential value for the region. In this way, poultry feed could become cheaper and more dependent on locally produced cassava. It is, therefore, recommended that 75% of maize in layers diet can be replaced with cassava meal of an appropriate ratio (65% tuber + 10% leaf) and the use of cassava should be encouraged not only to reduce dependence on maize, but also to reduce cost of feed in poultry industry.

Summary

The reduction of the hydrocyanic acid potential (HCNp) content of cassava foliage and tubers during processing processes and cultivation management were clarified and then evaluate the feeding values as feed for poultry.

1. Comparison of feed conditioning techniques to reduce cyanide contained in two varieties of cassava tuber

The effect of soaking and wilting processing methods on the HCNp contents of different varieties of cassava (Red and White) was evaluated. Although the initial HCNp contents of Red and White cassava varieties were different, the soaking and wilting methods could reduce the HCNp content for different varieties by similar mean reduction levels in HCNp contents. Moreover, the soaking method corresponds to 61.8-66.3% of HCNp reduction after 48 h meanwhile the wilting method corresponds to 50.9-51.5% in both cassava varieties. This investigation highlighted the importance of soaking cassava tuber at least 48 h prior to utilization as feed for animals.

2. Effect of ensiling process and additive effects of fermented juice of epiphytic lactic acid bacteria on the cyanide content of two varieties of cassava

The effect of ensiling process on reduction of the HCNp content of cassava leaves and tuber with fermented juice of epiphytic lactic acid bacteria (FJLB) as silage additive

was evaluated. In spite of the different cassava varieties used for processing in ensiling process, similar mean reduction levels (72.3-84.3%) in HCNp content were obtained on the 14 d after ensiling of FJLB treated silage. The low levels of HCNp content (25.1-82.3 mg/kg) in both leaves and tuber of Red cassava and (18.5-74.0 mg/kg) in White cassava were observed in the FJLB additive treatments on the 14 d after ensiling. Moreover, the relation between the drop of pH with silage fermentation and the reduction of HCNp concentration were shown to be inversely proportional each other. In addition, the addition of FJLB to cassava silage ensured good quality silage as indicated by high lactic acid bacteria counts and V-score value on 14 d after ensiling period.

3. Strategies for reducing of cyanogens in cassava and improving foliage and tuber yield by fertilizing

The effect of different fertilization rates with nitrogen (N) and potassium (K) combination on the chemical composition including HCNp and yielding of cassava was determined. The minimum HCNp content in cassava foliage and tuber were obtained by the application of N50-K_2O100 followed by N50-K_2O250 kg/ha. The foliage yield (2930.9 kg/ha) peaked at N50-K_2O100, but the tuber yields (9474.1 kg/ha) obtained at N50-K_2O250. As a consequence with the yields, the high CP yields in the foliage and

tuber were obtained in N50-K_2O100 and N50-K_2O250 treatments. Therefore, N50 -K100~250 combination was recommended for the optimum economical dose for the purpose of lower HCNp content of cassava for safe utilization.

4. Effects of harvesting period on chemical composition and yielding of cassava foliage and tuber

The effect of foliage harvesting periods on the chemical composition including HCNp and yielding of tuber was investigated. Two different ages of cassava foliage of initial harvesting, at 3 and 5 months were conducted for IH3 + FH and IH5 + FH treatments, respectively. The final harvests of these two treatments were done the whole including tuber in the 7 month. Cassava foliage, harvested once at root harvest (7 month), was performed as a control treatment (FH). The lowest HCNp content of cassava foliage was recorded in the FH treatment while the highest was observed in the IH3 + FH treatment. The highest total foliage yield was observed at IH5 + FH (3721.7 kg/ha) compared with IH3 + FH (3209.5 kg/ha) and FH (3426.9 kg/ha). Therefore, it is suggested to harvest at IH5 + FH system because the young cassava foliage obtained as sources of protein together with acceptable level of tuber yield.

5. Effects of cassava substitute for maize based diets on performance characteristics and egg quality of laying hens

The effect of substituting maize in layer diets with cassava tuber and different levels of leaf on laying performance and egg quality was evaluated. The best replacement percentage is 40% cassava meal (30% tuber + 10% leaf) gave the best performance in terms of egg production and Haugh unit score. Cassava leaf should be added along with tuber in replacement of maize in layer diet because cassava leaf is an appreciable source of carotenoids especially lutein and it could be used for adequate carotenoids supply for acceptable egg yolk color. Moreover, supplementation with cassava leaf (10% and 20%) to tuber was efficient in lowering cholesterol contents.

6. Effect of whole cassava meal as substitutes for maize in the diets on the performance characteristics and egg quality of laying hens

The effect of complete replacement of maize with cassava on the performance and egg quality of laying hens was investigated. Diet I, contained no cassava meal and served as control. In diet II, III and IV, the proportion of maize was replaced with the cassava meal at the levels of 50% (40% tuber + 10% leaf), 75% (65% tuber + 10% leaf), and 100% (90% tuber + 10% leaf), respectively. The egg production was able to tolerate up to 75% of cassava meal after which it declined.

7. Conclusion

In this study, cassava can be cultivated by applying N:K_2O fertilization and appropriate HCNp reducing method such as silage making to produce low cyanide cassava which is safe to use as feed. Cassava meal obtained by appropriate cultivation management could be replaced up to 75% of maize in the commercial diet of laying hens. Moreover, supplementation of cassava leaf to tuber is new feed resources to meet the necessary nutrition and it can be substituted for grain such as maize in poultry feed.

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要 約

本研究では，飼料調製過程ならびに栽培管理におけるキャッサバ茎葉および塊根中HCNp含量の減少を明らかにすると共に，家禽用飼料としての飼料価値を評価した。

1．キャッサバ2系統における塊根中シアン低減のための飼料調製技術の比較

浸漬および予乾処理による2系統のキャッサバ(RedおよびWhite)中HCNp含量の低減効果を評価した。処理前のRedおよびWhite系統のキャッサバ中HCNp含量は異なる水準を示していたが，浸漬および予乾調整後の平均HCNp減少率は両系統で同等の値を示した。処理後48時間のHCNp減少率は，浸漬処理で61.8-66.3%，予乾処理では50.9-51.5%の範囲を示した。浸漬処理による減少率が予乾処理より高く，浸漬時間は24～48 h が最も高い減少率を示した。このため，キャッサバ塊根を家畜飼料として利用する場合，少なくとも48時間前の浸漬処理を行うことが推奨された。

2．サイレージ調製および付着乳酸菌発酵液の添加が 2 系統のキャッサバ中シアン含量に及ぼす影響

サイレージ調製がキャッサバ葉ならびに塊根中HCNp含量に及ぼす影響を検討し，併せて付着乳酸菌発酵液（Fermented juice of epiphytic lactic acid bacteria: FJLB）の添加効果を評価した。異なるHCNp水準のキャッサバ2系統でサイレージ調製を行ったが，貯蔵後14日目のFJLB添加サイレージにおけるHCNp低減率は両系統で同等の高い値を示した(72.3-84.3%)。貯蔵後14日目のFJLB添加サイレージにおける葉および

塊根のHCNp含量は，Redキャッサバで25.1-82.3 mg/kg，Whiteキャッサバでは18.5-74.0 mg/kgの範囲となり，低い水準となった。また，両キャッサバ系統においてサイレージ乳酸発酵に伴うpHの低下とHCNpの低減率との関係は互いに反比例することが示された。さらに，キャッサバサイレージの調製時にFJLBを添加することで，乳酸菌数の増加およびV-スコアの向上による良質なサイレージを確保できると考えられた。

3．キャッサバ中シアンの低減ならびに茎葉と塊根収量の向上のための施肥管理技術の検討

窒素（N）とカリウム（K）の異なる施肥割合がキャッサバ茎葉および塊根の収量，HCNpならびに栄養成分に及ぼす影響を検討した。キャッサバ茎葉および塊根中HCNp含量はN50-K_2O100 kg/ha の施肥割合で最も低い値を示し，続いて，N50-K_2O250 kg/haで低い値となった。茎葉収量はN50-K_2O100 kg/haで2930.9 kg/ha，塊根収量はN50-K_2O250 kg/haで9474.1 kg/haとなり，処理区内においてそれぞれ最も高い値を示した。乾物収量の増加に伴い，茎葉および塊根のCP収量はN50-K_2O100 kg/haならびにN50-K_2O250 kg/haの施肥割合で高い値を示した。このことから，キャッサバの安全利用と施用効率の観点からN50 kg - K_2O100~250 kg/haが最適なNおよびK施用量の組み合わせであると考えられた。

4．キャッサバ茎葉の収穫時期が茎葉および塊根の収量ならびに化学成分に及ぼす影響

キャッサバ茎葉の収穫時期が茎葉および塊根中 HCNp，栄養成分ならびに収量

に及ぼす影響を検討した。茎葉は植付け後 3 ヶ月目あるいは 5 ヶ月目にそれぞれ最初の収穫を行い，さらに植付け後 7 ヶ月目に最終収穫を行った(IH3 + FH 区 および IH5 + FH 区)。また，最終収穫時にのみ茎葉を収穫する対照区を設けた(FH 区)。全ての処理区において最終収穫時に塊根の収穫を併せて行った。キャッサバ茎葉中 HCNp 含量は FH 区で最も低い値を示し，IH3 + FH 区で最も高い値を示した。茎葉収量は IH5 + FH 区で 3721.7 kg/ha となり，IH3 + FH 区(3209.5 kg/ha)および FH 区(3426.9 kg/ha)と比較して最も高い値を示した。このことから，植付け後 5 ヶ月目での茎葉収穫体系(IH5 + FH 区)では，塊根収量の許容水準を維持しながら，タンパク源として若い茎葉を獲得することが可能であると考えられた。

5. 配合飼料中トウモロコシのキャッサバによる一部代替が採卵鶏の産卵成績と卵質に及ぼす影響

キャッサバ塊根および異なる水準の茎葉で配合飼料中トウモロコシを一部代替した飼料給与が採卵鶏の産卵成績ならびに卵質に及ぼす影響を検討した。キャッサバミール 40%（塊根 30%+茎葉 10%）代替区において産卵率とハウユニットスコアが最も高い値を示した。キャッサバ茎葉はカロテノイド，特にルテインの供給源として，嗜好性のある卵黄色とするために十分なカロテノイドの供給を可能とした。このため，採卵鶏用飼料中のトウモロコシをキャッサバ塊根で代替する場合，茎葉を併用することが推奨される。さらに，キャッサバ茎葉 (10%および 20%)の併用は卵黄中コレステロール値の低下に効果的であると考えられた。

6. 配合飼料中トウモロコシのキャッサバによる完全代替が採卵鶏の産卵成績と卵質に及ぼす影響

キャッサバミール（茎葉および塊根）で飼料中トウモロコシを完全代替した飼料給与が採卵鶏の産卵成績と卵質に及ぼす影響を検討した。対照区としてキャッサバミール代替なしの DietⅠ，キャッサバミール代替区として代替割合 50%（塊根 40%+茎葉 10%），75%（塊根 65%+茎葉 10%）および 100%（塊根 90%+茎葉 10%）のそれぞれ DietⅡ，ⅢおよびⅣを設けた。産卵成績は 75%までのキャッサバ代替区で維持することが可能であったが， 100%の代替区では低下傾向を示した。

7. 本研究のまとめ

本研究では，キャッサバ利用上の制限要因となっている非栄養的成分であるシアン化合物 HCNp 濃度を低減するため，従来法に加え，牧草由来の付着乳酸菌発酵液を添加したサイレージ乳酸発酵で処理する新たな方法と比較したところ，サイレージで最も高い低減率が得られた。次に，栽培する上での肥培管理について検討し，ヘクタール当り，窒素 50kg，カリウム 100～250kg で高い収量と共に，最も低い HCNp 濃度となった。また，植付けから最終収穫の約2か月目前までに，地上部の茎葉を収穫確保することで，地下部の塊根と併せて，最も高い乾物とタンパク質収量が得られた。これらの結果に基づき，飼料調製した塊根と，従来，未利用資源であった茎葉によって，市販の配合飼料中のトウモロコシを代替した，家禽用飼料の給与が，採卵鶏の産卵成績と卵質に及ぼす影響を検討し，塊根 30%と茎葉 10%の代替区で高い産卵成績と優

れた卵質が得られた。また，産卵成績から，トウモロコシ 75%までの代替を可能とした。本研究の結果から，キャッサバの飼料資源としての安全性の確保を考慮した，効果的な栽培管理と調製方法を明らかにされ，トウモロコシ等の穀実飼料原料との代替を可能とする知見が得られた。