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Effects of Cassava Substitute for Maize Based Diets on Performance Characteristics and Egg Quality of Laying Hens

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Abstract: The effects of feeding diets containing cassava meal (tuber and leaf) were investigated on feed intake, feed conversion ratio, weight gain, egg performance and egg quality of layer during 5 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 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. Egg laying rate tended to decrease with the IV, although there was no significant different between them. Haugh unit value in the hens fed diet III were significantly higher ($p<0.05$) than those fed other diets. Hens fed cassava meal diets had higher yolk color score than the control fed with maize based diet. The results demonstrated that, up to 40% of maize could be replaced with cassava meal for improving laying performance and egg quality. Moreover, supplementation with cassava leaf to tuber was efficient in lowering yolk cholesterol contents.

Key words: Cassava, cholesterol, egg quality, laying hen, yolk color

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). 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.

The use of cassava as an alternative to conventional energy feedstuffs like maize could help to reduce feed costs (Anaeto and Adighibe, 2011). However, cassava contains various levels of cyanogenic glucosides mainly as linamarin and to a lesser extent as lotaustralin that are present in the leaf and tuber of the plant (Agbor-Egbe and Mbome, 2006). The low protein content, essential vitamin and minerals of cassava tubers have been the major factors limiting its use in poultry diets. Oruwari *et al*. (2003) stated that with proper protein balance, cassava meal could completely replace maize in poultry diets. According to composition values, cassava leaves are rich in protein, minerals, vitamins and all essential amino acids except methionine and tryptophan (Eggum, 1970). 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 and egg quality parameters.

MATERIALS AND METHODS

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 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 1. Four experimental diets were

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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 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.

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.

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

Table 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 determined

Table 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	17.55	17.57	18.07
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 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).

Laboratory analyses: The proximate composition and detergent fiber components of the diets were determined by using the methods 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.

Statistical analysis: All data collected were subjected to analysis of variance 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 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 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 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 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

Table 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±2.27	98.40±2.04	96.48±2.23	101.29±1.42
Egg production (%)	98.33±0.66	97.00±0.58	97.67±0.33	96.33±1.76
Egg weight (g)	57.52±1.24	58.81±0.92	56.72±0.83	59.39±0.65
FCR (kg feed/kg egg)	1.71±0.42	1.71±0.21	1.76±0.04	1.78±0.04
Live weight gain (kg)	0.06±0.02	0.10±0.06	0.04±0.06	0.08±0.07
Mortality (%)	-	-	-	-

FCR; Feed conversion ratio. Values (mean±SE) were not significantly different ($p>0.05$) among the treatments

Table 4: Egg quality parameters of laying hens fed experimental diets

Parameters	Diet I	Diet II	Diet III	Diet IV
Egg shape index	$73.57±0.47^{b}$	$73.97±0.51^{b}$	$74.43±0.61^{ab}$	$75.92±0.69^{a}$
Yolk index	0.40±0.00	0.40±0.00	0.40±0.00	0.40±0.01
Yolk weight (g)	15.59±0.55	16.48±0.44	15.38±0.25	16.15±0.23
Haugh unit	$89.17±0.95^{b}$	$88.46±0.81^{b}$	$92.45±1.07^{a}$	$88.74±1.10^{b}$
Egg shell weight (g)	5.41±0.09	5.51±0.14	5.54±0.13	5.72±0.81
Egg shell strength (Kgf)	4.68±0.10	4.45±0.19	4.55±0.18	4.61±0.16
Egg shell thickness (mm)	0.44±0.01	0.44±0.01	0.45±0.00	0.45±0.01

^{a,b}Values (mean±SE) with different superscript on the same row are significantly different ($p<0.05$)

Table 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±0.36^c	12.67±0.17^a	11.78±0.15^b	11.89±0.11^b
Lightness, L*	59.79±0.37^a	55.22±0.27^c	56.37±0.30^b	56.19±0.31^b
Redness, a*	7.50±0.17^c	13.44±0.28^a	11.67±0.23^b	11.75±0.27^b
Yellowness, b*	50.23±0.52^a	44.39±0.46^c	46.08±0.32^b	45.55±0.36^{bc}

$^{a-c}$Values (mean±SE) with different superscript on the same row are significantly different (p<0.05)

†Yolk color score obtained from digital egg tester (NABEL-DET-6000, Nabel Co. Ltd., Kyoto. Japan)

Table 6: Pigment composition of control diet, cassava leaf and tuber

Parameters	Diet I (control diet)	Cassava leaf	Cassava tuber
Beta-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 determined

Table 7: Pigment composition of egg yolk for eggs of dietary treatments

Parameters	Diet I	Diet II	Diet III
Vitamin A (retinol) (µg/100 g)	ND	1.00	1.00
Beta-carotene (µg/100 g)	ND	6.00	10.00
Lutein (mg/100 g)	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 determined

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 beta-carotene and lutein were not detected in cassava tuber. However, these pigments were observed in the eggs of cassava dietary treatments (Table 7). Vitamin A (retinol) and beta-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 (Fig. 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.

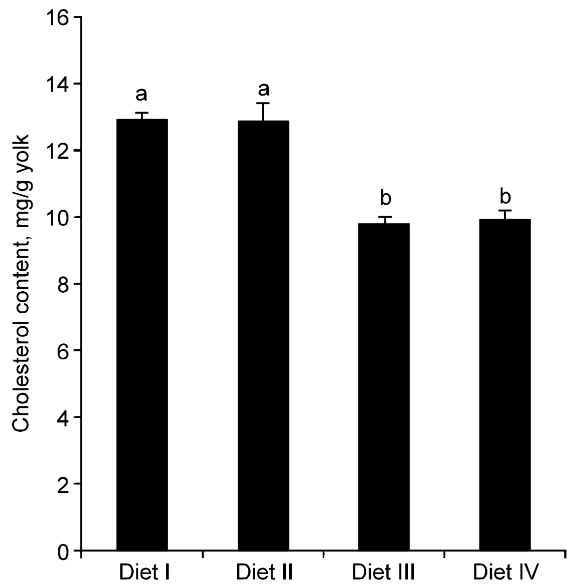

Fig. 1: Egg yolk cholesterol contents of laying hens fed experimental diets. Values are expressed as the mean±SE. ^{a,b}Means with different superscript are significantly different (p<0.05)

DISCUSSION

The CP content of cassava leaf was higher than that of maize and cassava tuber (Table 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 beta-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, beta-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 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 hypocholesterolemic influence as glycosides have ability to interfere with the intestinal absorption of the dietary cholesterol and lipid (Brown *et al.*, 1999).

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, beta-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. Therefore, 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.

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