**Effect of supplementary DL-methionine in pig diets with cassava leaves as a major protein source**

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**Abstract**

A feeding trial was conducted to evaluate the effect of different levels of  DL-methionine supplementation on growth performance of pigs given diets with cassava leaves and cassava root products providing 20 and 30% of the diet dry matter (DM). Twenty eight crossbred (Large White x Mong Cai) pigs with an average initial weight of 27.2  2.7 kg were housed in individual pens and allocated to 7 treatments in a  completely randomized design. Six treatments were arranged as a 2\*3 factorial the factors being fresh (FCL) or ensiled (RCL) cassava leaves and 0, 0.1 or 0.2% supplementary DL-methionine. The seventh treatment was the basal (control) diet which contained (% in DM) rice bran 29, maize 23, ensiled cassava root 20, cassava root meal 10 and fish meal 15. The 6 diets in the factorial arrangement were prepared by replacing 6 percentage units of fish meal and 14 percentage units of rice bran with either fresh or ensiled cassava leaves (20% in the diet DM), plus the indicated levels of DL-methionine. The experiment lasted 80 days.

Growth rates were higher, and DM and crude protein conversion rates tended to be better, for fresh compared with ensiled cassava leaves. Intakes of DM and crude protein were not affected by processing of the cassava leaves, nor by level of supplementary DL-methionine. Live weight gains and DM conversion improved linearly with level of supplementary DL-methionine. At slaughter, the weights of the liver and thyroids, the loin eye area and estimated lean meat yield were increased on fresh compared with ensiled leaves.  Estimated yield of lean meat increased and back fat decreased as the level of supplementary methionine was increased.

There were no differences in growth performance between the best diet from the cassava leaves-methionine treatments (FCL + 0.2% DL-methionine) and the control diet. Back fat thickness was less and estimated yield of lean meat was greater on the former diet.

**Keyword:** Carcass, conversion, ensiling, growth performance

**Efecto de la suplementación con DL-metionina en dietas de cerdos con hojas de yuca como una importante fuente de proteína**

**Resumen**

En este ensayo se evaluó el efecto de diferentes niveles de suplementación con DL-metionina en el crecimiento de cerdos alimentados con hojas de yuca y productos de raiz de yuca que suministraban el 20 y 30% de la materia seca (MS) de la dieta. Veintiocho cerdos cruzados (Large White x Mong Cai) con peso inicial promedio de 27.2 ± 2.7 kg alojados en corrales individuales se asignaron a 7 tratamientos en un diseño completamente al azar. Seis tratamientos se organizaron como un factorial 2\*3, siendo los factores: hojas de yuca frescas (FCL) o ensiladas (RCL), y suplementación con DL-metionina al 0, 0.1 o 0.2%. Al septimo tratamiento se le ofreció la dieta basal (control) que contenía (% de MS): salvado de arroz 29, maíz 23, raíz de yuca ensilada 20, harina de raíz de yuca 10 y harina de pescado 15. Las 6 dietas en el arreglo factorial fueron preparadas reemplazando 6 unidades en porcentaje de harina de pescado y 14 unidades en porcentaje de harina de arroz con hojas de yuca frescas o ensiladas (20% en la MS de la dieta), más el nivel indicado de DL-metionina. El experimento tuvo una duración de 80 días.

La tasa de crecimiento fue mayor, y la conversión de la MS y la proteína cruda mostraron tendencia a ser mejores, con las hojas de yuca fresca vs. las ensiladas. Los consumos de MS y proteína cruda no variaron por el procesamiento de las hojas de yuca, ni por el nivel de suplementación con DL-metionina. La ganancia de peso vivo y la conversión de la MS mejoró linealmente con el suplemento de DL-metionina. Al sacrificio, el peso del higado y la tiroides, el área de ojo de lomo y el rendimiento estimado de carne magra incrementaron en las dietas con hojas frescas de yuca vs. ensiladas. El rendimiento estimado de carne magra se incrementó y la grasa dorsal disminuyó a medida que el nivel de metionina suplementaria fue incrementado.

No se presentaron diferencias en crecimiento entre la mejor dieta con hojas de yuca-metionina (FCL + 0.2% DL-metionina) y la dieta control. El espesor de la grasa dorsal fue menor y el rendimiento estimado de carne magra fue mayor en la primera dieta.

**Palabras clave:**carcasa, conversión, ensilaje, crecimiento

**Introduction**

In Viet Nam, pig production plays a very important role at the household and at national level. Currently the pig population in Vietnam is estimated at 26 million animals (General Statistical Office 2004).  The majority of the pigs for slaughter are produced on small scale farms. However, under village conditions the use of protein supplements is limited because of the high cost. The animals are mainly fed with cheap local materials and unconventional feed resources in order to minimize the costs. Generally, conventional protein supplements such as soybean and fish meal are not used because they are expensive. This situation has given rise to a range of research reports concerning the feasibility of using protein-rich forages as a means of improving the nutritive value of traditional pig diets (Bui Huy Nhu Phuc 2006; Preston 2006).  Particular attention has been given to the use of cassava leaves as a protein source as these are widely available in Vietnam at farm level. The availability of this resource has increased with the changing role of cassava from being a “food crop” to an “industrial crop” for starch processing and the animal feed industry. At the present time about 330 000 ha are planted with cassava with a total yield of the order of 4 million tonnes of roots. The total cassava starch production in Vietnam is estimated atabout 500,000 tonnes, 70% of which is for export (Hoang Kim et al 2000).

Cassava is traditionally grown for the production of roots. However, the leaves have become increasingly important as a source of protein for monogastric and ruminant animals (Preston 2001; Wanapat 2001).  Cassava leaves are rich in protein but they are low in sulfur amino acids (Gomez et al 1985). The leaf protein is reported to be limiting in methionine and tryptophan but rich in lysine, with an overall biological value of 49 to 57% (Frochlich et al 2001). By the addition of synthetic methionine, the biological value of the protein could be increased to 80% according to Rogers and Milner (1963) and Eggum (1970). The advantages of adding methionine and cystine to pig diets based on cassava root have been demonstrated by Maner and Gomez (1973).

**Materials and methods**

**Animals and management**

Twenty-eight crossbred pigs (Mong Cai female x Large White male) aged about 80 days and with mean body weight of 27.2  1.7 kg (mean  SD).were housed in individual cages. They were vaccinated against hog cholera and pasteurellosis, and de-wormed 2 weeks before being randomly allocated to seven dietary treatments.

**Experimental design, diets and feeding**

The control diet consisted of rice bran, maize, cassava root meal, ensiled cassava root and was supplemented with  fish meal (FM) as the sole source of supplementary protein. The cassava root meal and ensiled cassava root provided 50% of the total diet dry matter (DM). The six test diets were formulated by replacing part of the fish meal of the control diet with either fresh or ensiled cassava leaves supplemented with zero, 0.1 or 0.2% DL-methionine (Table 1).

The cassava leaves were from a highly productive variety that is common in central Vietnam. They were chopped and wilted under a roof (fresh leaves) or ensiled for 90 days with 5 % molasses as described by Du Thanh Hang (1998).  The cassava roots were ensiled with 0.5% common salt (Nguyen Thi Loc et al 1996) and stored 21 days before feeding.

The diets were fed in amounts that led to minimum refusals with the cassava leaves given as a separate feed. Feeding times were 8:00, 10:00 and 15:00 h for the cassava leaves and 6:00, 12:00 and 17:00 h for the other components of the diets. The refusals were collected before each new meal was offered. Drinking water was freely available.

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| **Table 1.** Ingredients in the experimental diets (% DM basis) | | | | | | | |
|  | **Control** | **ECL** | **ECL-0.1M** | **ECL-0.2M** | **FCL** | **FCL-0.1M** | **FCL-0.2M** |
| Rice bran  Maize  ECR  CRM  Fish meal  FCL  ECL  Premix  DL-methionine  Soybean oil | 29.0  25.0  20.0  10.0  15.0  0  0  1.0  0  0 | 13.5  23.0  20.0  12.5  9.0  0  20.0  1.0  0  1.0 | 13.5  22.9  20.0  12.5  9.0  0  20.0  1.0  0.1  1.0 | 13.5  22.8  20.0  12.5  9.0  0  20.0  1.0  0.2  1.0 | 13.4  25.0  20.0  9.7  9.0  20.0  0  1.0  0  1.9 | 13.4  23.0  20.0  11.6  9.0  20.0  0  1.0  0.1  1.9 | 12.0  23.0  20.0  12.9  9.0  20.0  0  1.0  0.2  1.9 |
| *FCL: Fresh cassava leaves; ECL: Ensiled cassava leaves; CRM: Cassava root meal; ECR Ensiled cassava roots* | | | | | | | |

**Measurements**

*Feed intake and live weight*

Records were kept of amounts of feeds offered and refused. Live weights were taken every 20 days and growth rate calculated from the slope of the linear regression of weight against days on experiment.

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| **Table 2.** Chemical composition of the experimental diets (% in DM) | | | | | | | |
|  | **Control** | **ECL** | **ECL-0.1M** | **ECL-0.2M** | **FCL** | **FCL-0.1M** | **FCL-0.2M** |
| Crude protein | 14.2 | 14.4 | 14.5 | 14.5 | 14.7 | 14.7 | 14.6 |
| Lipids | 7.6 | 7.5 | 7.5 | 7.5 | 7.6 | 7.5 | 7.3 |
| Crude fiber | 3.8 | 6 | 6 | 6 | 5.9 | 5.9 | 5.8 |
| Ash | 6.9 | 6.3 | 6.3 | 6.3 | 6.4 | 6.4 | 6.3 |
| Ca | 0.63 | 0.6 | 0.6 | 0.6 | 0.68 | 0.68 | 0.68 |
| P | 0.63 | 0.51 | 0.51 | 0.51 | 0.53 | 0.52 | 0.51 |
| ME, MJ/kg# | 13.8 | 13.5 | 13.4 | 13.4 | 14.0 | 14.0 | 14.0 |
| Lysine# | 0.68 | 0.72 | 0.72 | 0.72 | 0.74 | 0.74 | 0.73 |
| Methionine# | 0.27 | 0.27 | 0.37 | 0.47 | 0.29 | 0.39 | 0.48 |
| Cystine# | 0.21 | 0.18 | 0.18 | 0.18 | 0.2 | 0.2 | 0.19 |
| HCN (mg/kg DM) | 27.8 | 72.7 | 72.7 | 72.7 | 97.2 | 98.5 | 99.4 |
| *# Estimated from literature values* | | | | | | | |

**Carcass measurements**

For the evaluation of carcass traits, 21 representative pigs (3/treatment) were starved for 24 hours and weighed prior to slaughter. Carcass traits were measured immediately after slaughter. The P2 back fat thickness was measured on the partitioned carcass 10 cm from the midline behind the 10th rib using a trace paper and a ruler; the loin area was measured by tracer paper.

**Chemical analyses**

Samples of feed and refusals were dried at 600C for 24 h and ground through a 1 mm sieve prior to chemical analysis according to the standard methods of AOAC (1984). Dry matter (DM) was measured by drying fresh samples at 1050C for 24 h. Crude protein was determined on fresh samples by the Kieldahl method (N\* 6.25). Ether extract (EE) was determined by Soxhlet extraction. Ash was the residue after ashing the samples at 6000C. Calcium and phosphorous were determined according to AOAC (1984) using the dry method and alkalimetric ammonium molybdophosphate method, respectively.

**Statistical analysis**

Analysis of variance was performed using the general linear model (GLM) procedure of Minitab Version 14. Two models were evaluated. The effects of processing of leaves of leaves (fresh versus ensiled)  and levels of methionine (0, 0.1 and 0.2%) were analysed as a 2\*3 factorial arrangement, the terms in the model being processing of cassava leaves, levels of methionine, the interaction processing of leaves\*methionine and residual error. The second model compared the best treatment from the six treatments in the 2\*3 factorial component of the experiment with the control.  The terms in this model were diet and error. When the data indicated linear relationships between performance traits and inputs (eg: levels of supplementary methionine), regression analysis was applied using the regression function in the Minitab software.

**Results**

**Growth and feed conversion**

*Main effects of processing of cassava leaves and supplementation with methionine*

There were significant effects on pig growth performance and carcass traits due to both cassava leaf processing and DL-methionine supplementation.  The interactions between the two factors were not significant, therefore the results are presented only for the main effects (Tables 3 and 5).

Growth rates were higher, and DM and crude protein conversion rates tended (P=0.1 and 0.18) to be better, for fresh compared with ensiled cassava leaves (Table 3). Intakes of DM and crude protein were not affected by processing of the cassava leaves, or by level of supplementary DL-methionine. . Live weight gains and DM conversion improved linearly with level of supplementary DL-methionine (Figures 1, 2 and 3).

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| **Table 3.** Mean values for main effects of ensiled versus fresh cassava leaves and levels of supplementary methionine (M) | | | | | | | | | | |
|  | **Ensiled** | **Fresh** | **SEM** | | **P** | **0.0M** | **0.1M** | **0.2M** | **SEM** | **P** |
| Live weight, kg | | | | | | | | | | |
| *Initial* | 26.8 | 27.6 | 0.93 | |  | 27.2 | 27.4 | 27.0 | 1.1 |  |
| *Final* | 72.6 | 78.5 | 1.2 | | 0.004 | 69.6 | 74.3 | 82.7 | 1.2 | 0.001 |
| *Daily gain* | 0.578 | 0.657 | 0.010 | | 0.001 | 0.540 | 0.599 | 0.712 | 0.012 | 0.001 |
| Feed intake, g/day | |  |  | |  |  |  |  |  |  |
| *Crude protein* | 284 | 299 | 8.3 | | 0.21 | 297 | 291 | 286 | 10 | 0.74 |
| *DM* | 1.97 | 2.05 | 0.057 | | 0.36 | 2.04 | 2.01 | 1.97 | 0.070 | 0.79 |
| Feed conversion | | | | | | | | | | |
| *Crude protein* | 0.496 | 0.466 | 0.015 | 0.18 | | 0.552 | 0.487 | 0.405 | 0.019 | 0.001 |
| *DM* | 3.45 | 3.19 | 0.11 | 0.10 | | 3.79 | 3.37 | 2.79 | 0.13 | 0.001 |

**Figure 1.** Relationship between live weight gain and level of supplementary DL-methionine  
 for pigs fed diets based on cassava roots and fresh or ensiled cassava leaves

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| **Figure 2.** Relationship between DM feed conversion and level of supplementary DL-methionine for pigs fed diets based on cassava roots and fresh or ensiled cassava leaves |  | **Figure 3.** Relationship between crude protein conversion and level of supplementary DL-methionine for pigs fed diets based on cassava roots and fresh or ensiled cassava leaves |

*Comparison with control diet*

There were no differences in performance traits between the control and the best of the treatments with cassava leaves and supplementary methionine (Table 4; Figures 4, 5 and 6).

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| **Table 4.** Mean values for growth performance traits of pigs fed  the best cassava leaves-methionine treatment (FCL-0.2M) and the control | | | | |
|  | **Control** | **FCL-0.2M** | **SEM** | **P** |
| Live weight | |  |  |  |
| *Initial* | 26.9 | 27.3 | 0.802 |  |
| *Final* | 85.1 | 86.8 | 0.865 | 0.232 |
| *Daily gain* | 0.749 | 0.772 | 0.0131 | 0.27 |
| Feed intake | |  |  |  |
| *DM* | 2.04 | 1.97 | 0.093 | 0.591 |
| *Crude protein* | 298 | 287 | 13 | 0.59 |
| Conversion | |  |  |  |
| *DM* | 2.73 | 2.56 | 0.141 | 0.434 |
| *Crude protein* | 0.397 | 0.373 | 0.0205 | 0.436 |

**Figure 4.**  Mean values for growth rate of pigs fed a control diet or the control diet with fresh or ensiled cassava leaves   
as partial replacement for fish meal each with 3 levels of supplementary methionine

**Figure 5.** Mean values for DM feed conversion  of pigs fed a control diet or the control diet with fresh or ensiled cassava leaves   
as partial replacement for fish meal each with 3 levels of supplementary methionine

**Figure 6.**  Mean values for crude protein conversion for pigs fed a control diet  or the control diet with fresh or ensiled cassava leaves   
as partial replacement for fish meal each with 3 levels of supplementary methionine

**Carcass traits**

*Main effects of processing of cassava leaves and supplementation with methionine*

The only traits affected by processing of the cassava leaves were the weights of the liver and thyroids, the loin eye area and estimated lean meat yield all of which were increased on fresh compared with ensiled leaves (Table 5). There was a suggestion that the liver and thyroids were lighter when DL-methionine was supplemented to the diets (P=0.06 and 0.03). Back fat decreased as the level of supplementary methionine was increased.

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| **Table 5**.  Mean values for main effects of ensiled versus fresh cassava leaves and levels of supplementary methionine (M) on carcass traits  (adjusted by covariance  for differences in slaughter live weight) | | | | | | | | | |
|  | **Ensiled** | **Fresh** | **SEM** | **P** | **0.0M** | **0.1M** | **0.2M** | **SEM** | **P** |
| Carcass |  |  |  |  |  |  |  |  |  |
| *Hot weight, kg* | 59.8 | 60.4 | 0.442 | 0.370 | 59.7 | 60.2 | 60.5 | 0.686 | 0.280 |
| *Trimmed, kg* | 54.2 | 54.8 | 0.472 | 0.380 | 53.8 | 54.5 | 55.1 | 0.650 | 0.570 |
| *Length, cm* | 88 | 89.11 | 0.930 | 0.416 | 90.3 | 87.3 | 88.1 | 0.135 | 0.294 |
| Liver, kg | 1.39 | 1.61 | 0.025 | 0.006 | 1.59 | 1.46 | 1.46 | 0.035 | 0.060 |
| Loin area, cm2 | 27.1 | 28.0 | 0.221 | 0.012 | 27.8 | 27.2 | 27.6 | 0.300 | 0.350 |
| Back fat, cm | 2.99 | 2.88 | 0.057 | 0.196 | 3.24 | 2.90 | 2.66 | 0.080 | 0.007 |
| Thyroid, g | 32.2 | 34.6 | 0.688 | 0.031 | 35.6 | 31.5 | 33.2 | 0.900 | 0.030 |
| Intestines, cm |  |  |  |  |  |  |  |  |  |
| *Small* | 15.0 | 14.9 | 0.358 | 0.813 | 15.7 | 14.4 | 14.7 | 0.500 | 0.229 |
| *Large* | 5.21 | 4.85 | 0.097 | 0.022 | 4.97 | 5.21 | 4.91 | 0.114 | 0.224 |

*Comparison with control diet*

The only differences in carcass traits between the control diet and the best of the cassava leaves – methionine diets was in back fat thickness,  which favored the experimental diet of fresh cassava leaves with 0.2% supplementary DL-methionine (Table 5).

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| **Table 6.** Mean values for carcass traits of pigs fed  the best cassava leaves-methionine treatment (FCL-0.2M) and the control | | | | |
|  | **Control** | **FCL-0.2M** | **SEM** | **P** |
| Carcass |  |  |  |  |
| *Hot weight, kg* | 67.0 | 65.8 | 0.43 | 0.162 |
| *Trimmed, kg* | 60.4 | 60.3 | 0.76 | 0.901 |
| *Length, cm* | 86.9 | 90.4 | 0.03 | 0.296 |
| Liver, kg | 1.49 | 1.62 | 0.039 | 0.131 |
| Loin area, cm2 | 28.3 | 28.7 | 0.30 | 0.509 |
| Back fat. cm | 3.70 | 2.86 | 0.070 | 0.005 |
| Thyroid, g | 35.0 | 35.8 | 0.32 | 0.145 |
| Intestines, cm |  |  |  |  |
| *Small* | 12.8 | 15.7 | 0.96 | 0.235 |
| *Large* | 3.98 | 5.02 | 0.53 | 0.333 |

**Discussion**

The noteworthy features of the results are: (i) the better growth performance of the pigs when cassava leaves were fed fresh rather than ensiled; and (ii) the marked improvement in growth rate (32%) and feed conversion (25%) due to DL-methionine supplementation, the response being similar for the diets with either fresh or ensiled cassava leaves.

Most of the earlier experiments with cassava leaves in pig diets have been predicated on the need to reduce the risk of toxicity from hydrocyanic acid, the precursors of which (cyanogenic glucosides) are present in high concentrations in the fresh leaves of most cassava varieties. Both ensiling and, to a greater extent, sun-drying have been shown to reduce HCN levels dramatically (Bui Huy Nhu Phuc 2006).  The surprising result in the present study was the better performance on the fresh compared with the ensiled leaves. As far as the authors are aware, there have been no direct comparisons between fresh and ensiled leaves in the same experiment. Chhay Ty and Preston (2005) fed fresh cassava leaves to young pigs at 50% of the DM of the diet, the energy component of which was broken rice, and reported no signs of HCN toxicity even though the levels of HCN (7.4 mg/kg live weight) exceeded what had been considered to be the thresh-hold level (3.5 mg/kg live weight) for toxicity (Tewe 1995). In the laboratory of the senior author (Du Thanh Hang et al 2006), pigs fed diets with 30% cassava root products and 20% cassava leaves (DM basis), had high DM intakes, which were not affected by processing method (washing chopping or wilting of the leaves). Intakes of HCN were from 6 to 15 mg/kg live weight, considerably above the recommended threshold for toxicity of 3.5 mg/kg live weight (Tewe 1995). In the present experiment HCN intakes were calculated to be 2.8 and 4.0 mg/kg live weight for the ECL and FCL diets, respectively, and were not related to growth performance.

A possible explanation for the poorer growth performance on ensiled versus fresh leaves could be the negative effects of the ensiling process on the biological value of the cassava protein. The fermentation taking place in ensiled forages converts soluble carbohydrates to organic acids and also promotes proteolysis (McDonald 1981). Oshima and McDonald (1978) reported that, in general, of the total N (TN) in fresh forages,75 to 90%  would be protein N (PN), and the remainder  non-proteinN (NPN) After ensiling, NPN mayaccount for as much as 80% of TN according to Papadopoulos and McKersie (1983)..  Owens et al (1999) found that when alfalfa and red clover were ensiled the NPN content increased from 136 to 353 (g/kg total N) in red clover and from 165 to 625 g/kg total N in alfalfa. There appear to be no comparable data on the ensiling of cassava leaves but it is probable there is some loss in protein quality during the ensiling process.

Positive effects of supplementary DL-methionine on growth performance of pigs fed cassava root meal were first reported by Maner and Gomez (1973). The use of cassava leaves together with cassava root products as the basis of pig diets is a more recent development. Nguyen Thi Hoa Ly (2006) observed a 23% increase in growth rate in pigs when DL-methione was added at 0.15% of  the DM of diets with 17 to 25% ensiled cassava roots and 15% ensiled cassava leaves (DM basis). By contrast, in the present study the growth rate increase was 32% and the levels of cassava roots and leaves were 30 and 20% respectively. In the experiment of Nguyen Thi Hoa Ly (2006) synthetic lysine was also added and was thus confounded with the level of methionine. Addition of 0.3% DL-methionine to a diet with 50% of the DM as fresh cassava leaves had no effect on growth rate or feed conversion of pigs fed a basal diet of broken rice (Chhay Ty and Preston 2005). In this case the contrast with the present study was in the basal diet which did not contain cassava root products. From a theoretical standpoint, it is to be expected that there would be nutritional benefits from DL-methionine supplementation of pig diets rich in cassava leaves because of the relative deficiency of the sulphur amino acids in cassava leaf protein and the fact that a source of sulphur is required for the detoxification of HCN.

**Conclusions**

* There were no differences in growth performance between the best diet from the cassava leaves-methionine treatments (FCL + 0.2% DL-methionine) and the control diet with 15% fish meal. Back fat thickness was less and estimated yield of lean meat was greater on the former diet.
* There is need for further research on the relative effects of fresh versus ensiled cassava leaves as major protein sources in pig diets as well as the potential benefits to be gained from supplementation with DL-methionine.

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