Protein-enrichment of cassava pulp as feed for growing pigs

Du Thanh Hang, Ho Le Quynh Chau, Le Thi Thu Hang and T R Preston[1]

Faculty of Animal Science and Veterinary Medicine, Hue University, Vietnam
duthanhhang@huaf.edu.vn
[1] Centro para la Investigación en Sistemas Sostenibles de Producción Agropecuaria (CIPAV), Carrera 25 No 6-62 Cali, Colombia

Abstract

In a solid-state fermentation of a mixture of cassava pulp-maize grain (70:30), with urea (2%) and diammonium phosphate (1.5%), the conversion of crude to true protein was better when the inoculum was *Bacillus subtilis* rather than *Aspergillus niger* and when the fermentation was for 14 rather than 21 days. The rate of conversion of substrate to true protein was 7.2 g DM per 1 g of true protein over the 14-day fermentation.

When the basal diet of growing pigs was replaced with protein-enriched cassava pulp responses in live weight gain and feed conversion were curvilinear with no improvements (live weight gain) and slight improvement (feed conversion) as the enriched pulp was increased to 25% of the diet DM, followed by deterioration in response with higher levels of enriched pulp.

We suggest that promoting studies on high biomass-producing plants such as *Colocasia esculenta (L) Schott* is a more appropriate way forward to produce high quality protein for animal feeding than solid state fermentation of carbohydrate that can best be used directly by the animals.

Key words: *Aspergillus niger, Bacillus siubtilis, digestibility, greenhouse gas, nitrogen retention, true-protein*

Introduction

Cassava plays an important socio-economic role as a secondary crop in Viet Nam. The cropping area of 570.000 ha produces some10 million tonnes of tubers that after processing for starch yield byproducts - cassava pulp and cassava root peel – accounting for about 45% of the original weight of tubers. Solid-state fermentation of

these byproducts with fungi, yeast, and bacteria has been shown to improve the true protein content (Hong and Ca 2013; Manivanh et al 2016; Vanhnasin and Preston 2016a; Sengxayalth et al 2017a). However, the overall conversion rate of substrate to true protein has not been reported and attempts to produce a balanced diet for pigs by this process have not been successful (Vanhnasin and Preston 2016b; Sengxayalth et al 2017b; Manivanh et al 2018b; Hong et al 2917).

The objective of this study was to develop a feeding system for growing pigs by fermenting a mixture of 70% cassava pulp and 30% maize with two micro-organisms - *Aspergillus niger, Bacillus subtilis* - in combination or separately, with supplementary nitrogen and phosphorus from urea and diammonium phosphate.

Location

Two experiments were carried out in Hue University in Viet Nam from October 2017 to March 2018.

Experiment 1: Fermentation of cassava pulp with *Aspergillus niger* and *Bacillus subtilis*

Materials and methods

Treatments and design

Aspergillus niger and Bacillus subtilis were used to ferment a mixture of cassava pulp and maize grain (70:30 DM basis), with 2% urea and 1.5% diammonium phosphate (DAP), with 3 replications of each organism employed separately or in combination. The fermentation was for 21 days with measurements taken at 0, 3, 7, 14 and 21 days. The inoculum of microorganisms was prepared by the procedure described by Hang and Quynh Chau (2018).

The DAP, urea and carbohydrate substrate were added to the suspensions of the microorganisms and placed in trays at a depth of approximately 5 cm for fermentation at room temperature during periods of 0, 3, 7, 14 and 21 days. After each period of fermentation, measurements were made of crude and true protein according to AOAC (2016). The data were analysed as a 3*6 factorial using the GLM program in the Minitab (2016) software.

Results

For individual and combined micro-organisms there were no advantages in extending the fermentation beyond 14 days (Table 1). Fermentation with *B. subtilis* supported a higher rate of conversion of crude to true protein than *A. niger* with intermediate results from the combination of the two micro-organisms.

Table 1. Effect of source of fermenting micro-organism and duration of fermentation on true protein (TP), crude protein (CP), ratio of TP/CP and crude fiber (CF) (as % in DM)

	TP	**CP**	**TP/CP**	**DM**	**CF**
A.niger	8.95b	13.5b	65.8b	75.4a	26.2a
A+B	10.a	14.4a	71.87a	76.8a	23.4c
B.sub	11.7a	14.3a	74.56a	77.1a	25.1b
SEM	0.13	0.09	1.123	0.697	0.089
p	<0.001	<0.001	<0.001	0.22	<0.001
Fermentation, days					
0	7.33d	12.87d	56.98d	86.4a	26.7a
3	9.35c	13.52c	68.98c	82.6b	26.0a
7	10.5b	14.21b	73.75b	74.90c	24.8b
14	11.7a	14.81a	79.15a	72.2c	23.5c
21	11.2ab	14.92a	74.9b	65.9d	21.8d
SEM	0.11	0.111	1.45	0.627	0.116
p	<0.001	<0.001	<0.001	<0.001	<0.001

[ab] *Means within main treatments without common superscript differ at p<0.05*

Table 2. Changes in the composition of the substrates during 21 days fermentation (data are overall mean values for the Ag, Bs and Ag-Bs treatments, derived from the data in Table 1)

Days	**DM, g**	**TP, g**	**CP, g**	**TP/CP**	**CF**
0	86.4	6.33	11.1	57.0	23.1
3	82.6	7.72	11.2	69.2	21.5
7	74.9	7.86	10.6	73.9	18.6
14	72.2	8.45	10.7	79.0	17.0
21	65.9	7.38	9.83	75.1	14.4

After 14 days of fermentation, 14.2 g of substrate had been used to produce an additional 2.12 g of true protein (Table 3; Figure 1), a conversion of 6.7 g substrate per 1g of true protein. It would appear that the fermenting organisms used almost equally the fiber and the non-fiber components in the substrate for protein synthesis.

Table 3. Balance of DM, crude protein and true protein before and after 14 days fermentation of 100g of substrate

	DM, g	**CP, g**	**TP, g**
Begin	86.4	11.1	6.33

After 14d	72.2	10.7	8.45
Gain/loss	-14.2	-0.4	2.12

Figure 1. Changes in concentration of dry matter and true protein in substrates with length of fermentation

The negative feature of the fermentation is that the other end-product will have been carbon dioxide, resulting in an overall negative carbon footprint for the process.

Experiment 2: Partial replacement of a conventional pig-fattening diet with protein-enriched feed derived from fermented cassava pulp-maize-rice bran

Materials and methods

The combination of *Aspergillus niger* and *Bacillus subtilis* with14 days of fermentation *was* chosen to produce the protein-enriched feed for the pig growth experiment (Table 4).

Table 4. Ingredients in the fermentation of cassava pulp/maize/rice bran (% in DM)

Cassava pulp	75
Maize	11.5
Rice bran	10
Mixture B. *subtilis* and *A. niger*	5
DAP	1.5
Urea	2

The fermentation was done daily in quantities sufficient to ensure the availability of the protein-enriched feed throughout the feeding trial.

Experimental design

The four treatments in a random block design with 4 replicates were:

PEC0: Control diet with no protein-enriched supplement

PEC12.5: The control diet with 12.5% of PEC (DM basis)

PEC25: The control diet with 25% of PEC

PEC50: The control diet with 50% pf PEC

Animals, housing and feeds

Sixteen castrated male (Mong Cai x Large White) pigs (24.5± 0.5 kg) were housed individually in metabolism cages. After 45d (pigs were 54±0.6 kg), feces and urine were collected over 5 days for measurement of digestibility and N retention. The growth experiment was continued to 80 days. Feed offered and refused and liveweights were recorded.

Table 5. Chemical **c**omposition of the ingredients of feeds (% in DM except for DM which is on air-dry basis)

Feed	**DM**	**CP**	**CF**
Rice bran	86.0	11.0	5.96
Maize	87.0	8.5	2.6
Fish meal	87.0	45.0	3.2
PEC	39.0	13.6	17.6

PEC : Protein-enriched cassava pulp/maize/rice bran

Table 6. Composition of experimental diets (% in DM)

	PEC0	**PEC12.5**	**PEC25**	**PEC50**
Maize	39.6	35.2	28.7	18.6
Rice bran	39.9	34.8	29.8	17.9
Fish meal	20.0	17.0	16.0	13.0
PEC	0.0	12.5	25	50
Premix Min-Vit	0.5	0.5	0.5	0.5
Compositión, % in DM				
CP	16.4	16.1	16.1	16.0
TP#	16.4	15.8	15.6	15.0
CF	4.8	6.2	7.3	9.1

[#] *Based on 80% of protein in PEC is true protein (experiment 1)*

Chemical analysis

DM, crude and true protein and crude fiber were determined following AOAC (1997) procedures.

Statistical analysis

The data were analyzed according to the GLM option in the ANOVA program of the Minitab (2016) software. Sources of variation were diets and error.

Results

Responses in live weight gain and feed conversion to inclusion of the protein-enriched feed were curvilinear with no improvement (LW gain) or slight improvement (feed conversion) as the protein-enriched feed provided up to 25% of the diet DM followed by deterioration in response, for both criteria, with 50% replacement by PEC (Table 7: Figures 1 and 2).

Table 7. Mean values for feed intake, live weight gain and feed conversion in pigs fed protein-enriched cassava pulp replacing up to 50% of the basal diet

	PEC0	**PEC12.5**	**PEC25**	**PEC50**	**SEM**	***p***
Live weight, kg						
Initial	24.8	24.1	24.6	24.1	0.486	0.63
Final	78.6[a]	79.1[a]	79.2[a]	72.9[b]	0.011	0.01
Daily gain	0.67[a]	0.6[a]	0.6[a]	0.61[b]	0.08	0.02
DM intake, kg/d	1.74[ab]	1.72[b]	1.69[b]	1.79[a]	0.33	0.04
FCR#	2.6[b]	2.50[b]	2.47[b]	2.95[a]	0.05	0.001

#DM intake /LW gain *[ab] Means without common superscript differ at p<0.05*

Figure 2. Curvilinear response in growth rate of pigs fed protein-enriched cassava pulp replacing up to 50% of the basal diet

Figure 3. Curvilinear response in feed conversion enriched cassava pulp replacing up to 50% of

Apparent digestibility of organic matter and crude protein did not differ among diets, but that for crude fiber showed a linear decrease as the protein-enriched feed in the diet was increased (Table 8).

Table 8. Mean values for DM intake and digestibility coefficients in pigs fed increasing levels of protein-enriched cassava pulp-maize-rice bran

	PEC0	**PEC12.5**	**PEC25**	**PEC50**	**SEM**	***p***
DMI, kg/d	1.63	1.5	1.74	1.7	0.08	0.172
Digestibility, %						
CP	67.2	74	71	68	2.37	0.158
OM	64	63	64	66	2.39	0.82

CF	28[a]	26[a]	23[b]	19[c]	2.15	0.01

[ab] *Means without common superscript differ at p<0.05*

N retention, expressed on daily basis, or as percent of N digested, decreased linearly as the proportion of protein-enriched feed in the diet was increased (Table 9; Figures 4 and 5).

Table 9. Mean values for N-balance in pigs fed creasing levels of protein-enriched cassava pulp-maize-rice bran

	PEC0	**PEC12.5**	**PEC25**	**PEC50**	**SEM**	***p***
N balance, g/d						
Intake	43.2	36.8	38.4	38.4	1.76	0.242
Feces	14.6	10.1	11.2	12	1.28	0.098
Urine	5.92	6.88	7.68	8.48	0.8	0.07
Retention	22.3[a]	20.5[ab]	19.7[ab]	17.6[b]	0.956	0.004
Ret%digN	77.9[a]	76.7[ab]	72.4[ab]	66.7[b]	1.69	0.001

[ab] *Means without common superscript differ at p<0.05*

Figure 4. Linear decrease in N retention in pigs fed protein-enriched cassava pulp replacing up to 50% of the basal diet

Figure 5. Linear decrease in N retention as per protein-enriched cassava pulp replacing up

Discussion

Developing alternatives to imported soybean meal for the pig industry is a laudable activity in tropical countries. The ready availability of carbohydrate-rich by-products from cassava roots processed for starch production has been the incentive to develop solid-state fermentation systems to promote growth and reproduction of micro-organisms such as yeast and the fungal strains studied in the present paper than can use ammonia-nitrogen for this purpose. The results of the experiments described in this paper, together with those from many other researchers, prove that the process is

feasible. The problem has been to devise a feeding system in which the "protein-enriched" feeds, developed with this process, can satisfactorily replace conventional protein sources such as fish and soybean meals.

The results from the feeding trial described in Experiment 2 confirm those from other sources -- that protein-enriched feeds produced by solid-state fermentation can replace no more than 20-30% of the dietary protein supply, beyond which performance deteriorates to a point that is not economical. In most cases the decline in performance is caused by a reduction in feed intake. As most observations indicate that the maximum degree of conversion-of added NPN sources to true protein is of the order of 70-80%, the obvious conclusion is that the residual NPN is the factor leading to reduced intake and therefore growth rate. This point has not been adequately studied. Manivanh et al (2018a) found no residual ammonia after yeast-fermentation of cassava root meal. They suggested that the incomplete conversion of urea-N and ammonia-N to yeast protein was because of incomplete hydrolysis of urea to ammonia due to action of urease being inhibited by the fall in pH during the fermentation.

The other issue raised by the present research is the efficiency of conversion of substrate carbohydrate to microbial protein. The results from experiment 1 indicate that it requires about 7 g of carbohydrate to produce 1 g of microbial protein using the mixed fungal strains of *B. subtilis* and *A. niger*. Manivanh et al (2016) reported a similar ratio using yeast as the fermentative organism. The related issue is that the byproduct of the fermentation process is carbon dioxide the presence of which in the atmosphere causes global warming. This leads to the question of the choice of system to produce the required protein. The alternative way is by growth of protein-rich plants which absorb atmospheric carbon dioxide as their source of energy and carbon-rich structure, as opposed to producing it as a byproduct of metabolism as in the case of protein produced by microbial growth. We suggest that promoting studies on high biomass-producing plants such as *Colocasia esculenta (L) Schott* (Hang et al 2018) is a more appropriate way forward to produce high quality protein for animal feeding than solid state fermentation of carbohydrate that can best be used directly by the animals.

Conclusions

- In a solid-state fermentation of a mixture of cassava pulp-maize grain (70:30), with urea (2%) and diammonium phosphate (1.5%), the conversion of crude to true protein was better when the inoculum was *Bacillus subtilis* rather than *Aspergillus niger* and when the fermentation was for 14 rather than 21 days.

- The rate of conversion of substrate to true protein was 7.2 g DM per 1 g of true protein over the 14-day fermentation.

- When the basal diet of growing pigs was replaced with protein-enriched cassava pulp responses in live weight gain and feed conversion were curvilinear with no improvements (live weight gain) and only slight improvement (feed conversion) as the enriched pulp was increased to 25% of the diet DM, followed by deterioration in response with higher levels of enriched pulp.

- We suggest that promoting studies on high biomass-producing plants such as *Colocasia esculenta (L) Schott* is a more appropriate way forward to produce high quality protein for feeding to pigs than solid state fermentation of carbohydrate that can best be used directly by the animals.

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