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EFFECT OF MULTI-ENZYME SUPPLEMENTATION ON ENERGY CONCENTRATION, NUTRIENT AND FIBER DIGESTIBILITIES AND GROWTH PERFORMANCE OF NURSERY PIGS FED DIETS WITH CASSAVA MEAL

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ABSTRACT

Two experiments were conducted to determine the effect of multi-enzyme supplementation on energy concentration, nutrient and fiber digestibilities and growth performance of nursery pigs fed diets with cassava meal (CM). In Exp. 1, 20 barrows (initial BW=8.23 ± 0.70 kg, PIC L337×C24) were randomly allotted to 1 of 4 dietary treatments with 5 replicates per treatment. Dietary treatments were a basal diet, 60% basal diet + 40% CM with no enzyme or supplemented with either 60 mg/kg of a multi-enzyme containing endo-1,4-ß-xylanase and endo-1,3(4)-ß-glucanase or 110 mg/kg of a multi-enzyme containing endo-1,4-ß-xylanase, endo-1,3(4)-ß-glucanase, and a 6-phytase. In Exp. 2, 378 pigs (initially weighing 6.6 ± 1.7 kg; PIC L337×C24) were randomly allotted to 1 of 3 dietary treatments using a randomized complete block design. Dietary treatments were a control diet and a corn-soy diet with 20% CM with and without 200 mg/kg of a multi-enzyme containing endo-1,4-ß-xylanase and endo-1,3(4)-ß-glucanase. Each treatment had 6 replications with 21 pigs per replicate. Results of the two experiments showed that multi-enzyme supplementation did not improve the ME of CM, digestibility of nutrients and dietary fiber and growth performance of nursery pigs. Therefore, the multi-enzymes used in this study do not affect the feeding value of diets containing CM.

Key words: cassava meal, energy, enzyme, growth performance, nursery pigs

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) meal is an excellent source of energy for swine as it contains 60 to 80% starch (Gomez, 1991). It is produced either from unpeeled or peeled cassava roots, which results in high variability in nutrient and crude fiber concentration (Aina and Fanimo, 1997; Kubkomawa *et al*., 2013). Previous research that measured the energy concentration in cassava meal is limited especially for nursery pigs; however, recent research in broilers indicated that the dietary fiber concentration of cassava meal negatively affects its metabolizable energy concentration (Masilungan, 2016). It is, therefore, hypothesized that the use of exogenous enzymes targeting the fiber fraction in diets containing cassava meal

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may improve its energy value and increase its utilization in swine diets.

The main non-starch polysaccharide (NSP) components of cassava meal are cellulose and non-cellulosic polysaccharides rich in arabinose and xylose (Bach Knudsen, 1997). Recently, new multi-enzyme products containing endo-1,4-ß-xylanase and endo-1,3(4)-ß-glucanase became available for use in swine diets and were developed primarily to hydrolyze pentosans and ß-glucans in different types of cereals. There is, however, no information on the effectiveness of these multi-enzyme combinations in diets containing cassava meal especially in nursery pigs. Therefore, the objective of this study was to determine the effects of multi-enzyme supplementation on energy concentration, nutrient and fiber digestibilities and growth performance of nursery pigs fed diets with cassava meal.

MATERIALS AND METHODS

Exp. 1. Energy Balance Study

Twenty weanling barrows (PIC 337×C24; initial BW = 8.23 ± 0.70 kg) were blocked by initial weight and randomly allotted to one of four dietary treatments using a randomized complete block design. There were five replicate pigs per treatment. Pigs were placed in individual metabolic cages (0.6 × 2.2 × 1.2 m) equipped with a feeder and a drinker, fully slatted floor, a screen floor, and urine trays which allowed for the total, but separate collection of feces and urine materials from each pig.

A corn-soy basal diet (CS) consisting of 77.51% corn and 18.86% soybean meal (as-fed basis) and vitamins and minerals was formulated (Table 1). The next diet was formulated by mixing 60% of the CS diet with 40% (as-fed basis) cassava meal (CM). The last two diets were the CM diet supplemented with either 60 mg/kg of a commercial multi-enzyme product containing 25,000 visco unit/g of endo-1,4-ß-xylanase, and 17,200 visco unit/g of endo-1,3(4)-ß-glucanase and 17 other enzyme activities, obtained from a fermentation broth of *Talaromyces versatilis* (NSPase; Rovabio Advance P, Adisseo, France) or 110 mg/kg of a commercial multi-enzyme product containing 6,250 visco unit/g of endo-1,4-ß-xylanase, 4,300 visco unit/g of endo-1,3(4)-ß-glucanase, 2,500 FTU/g of 6-phytase and 17 other enzyme activities, obtained from a fermentation broth of *Talaromyces versatilis* and *Schizosaccharomyces pombe* (NSPase+Phytase; Rovabio Max Advance P, Adisseo, France). All the experimental diets were in meal form.

Each pig was weighed at the beginning of the experiment. The quantity of feed provided per pig daily was calculated at 3 times their estimated requirement for maintenance energy (e.g. M = 106 kcal ME × $BW^{0.75}$; NRC, 2012) and divided into 2 equal meals (0700 and 1600 h). Water was made available at all times. The experiment lasted for 10 d. For the first 5 d, pigs were allowed to adapt to their diet, cage and environment. The next 5 d was the collection period. Feces and urine were collected from d 6 to d 10 using the marker-to-marker approach (Adeola, 2001). Urine was collected over a preservative of 50 ml of 6N HCL.

At the conclusion of the experiment, urine samples were thawed and mixed within animal and diet, and a sub-sample was lyophilized and used for analysis. Fecal samples were dried in a forced-air oven and finely ground prior to analysis. Fecal, urine, diet, and ingredient samples were analyzed in triplicate for GE using bomb calorimetry (Model 6300, Parr Instruments, Moline, IL). Diet and ingredient samples were analyzed in triplicate for

Table 1. Composition of experimental diets (as-fed basis), Exp. 1[1].

Ingredients	Diet	
	Corn-Soy Basal Diet	Cassava Meal
Corn	77.51	46.50
Soybean meal	18.86	11.32
Cassava meal	--	40.00
Monocalcium phosphate	2.57	1.54
Limestone	0.13	0.08
Vitamin-mineral premix[2]	0.36	0.22
Salt	0.57	0.34
Total	100.00	100.00

[1]Two additional treatments were prepared where 60 mg/kg of a commercial multi-enzyme product containing 25,000 visco unit/g of endo-1,4-ß-xylanase, and 17,200 visco unit/g of endo-1,3(4)-ß-glucanase and 17 other enzyme activities, obtained from a fermentation broth of *Talaromyces versatilis* (NSPase; Rovabio Advance P, Adisseo, France) and 110 mg/kg of a commercial multi-enzyme product containing 6,250 visco unit/g of endo-1,4-ß-xylanase, 4,300 visco unit/g of endo-1,3(4)-ß-glucanase, 2,500 FTU/g of 6-phytase and 17 other enzyme activities, obtained from a fermentation broth of *Talaromyces versatilis* and *Schizosaccharomyces pombe* (NSPase+Phytase; Rovabio Max Advance P, Adisseo, France) where supplemented to the cassava meal diet.
[2]Provided the following quantities of vitamins and micro minerals per kg of complete diet: Vitamin A, 11,128 IU; vitamin D3, 2,204 IU; vitamin E, 66 IU; vitamin K, 1.42 mg; thiamin, 0.24 mg; riboflavin, 6.58 mg; pyridoxine, 0.24 mg; vitamin B12, 0.03 mg; D-pantothenic acid, 23.5 mg; niacin, 44 mg; folic acid 1.58 mg; biotin, 0.44 mg; Cu, 10 mg as copper sulfate; Fe, 125 mg as iron sulfate; I, 1.26 mg as potassium iodate; Mn, 60 mg as manganese sulfate; Se, 0.3 mg as sodium selenite; and Zn, 100 mg as zinc oxide.

proximate composition (AOAC, 2007). Fecal, diet, and ingredient samples were analyzed in triplicate for NDF (Holst, 1973) and ADF (method 973.18; AOAC, 2007). Fecal, diet and ingredient samples were also analyzed for Ca and P using an atomic absorption spectrophotometer (methods 4.8.03 and 3.4.11, respectively; AOAC, 2007). Fecal samples and 20% of the collected urine were stored at -20°C immediately after collection.

The amount of energy lost in the feces and in the urine and the quantities of DE and ME in each of the diets were calculated (Adeola, 2001).

$$DE_{diet} = \frac{GE_{intake} - Fecal\ energy_{output}}{ADFI}$$

$$ME_{diet} = \frac{GE_{intake} - \left(Fecal\ energy_{output} + Urine\ energy_{output}\right)}{ADFI}$$

The apparent total tract digestibility (ATTD, %) of DM, OM, GE, CP, crude fat, ash, NDF, ADF, Ca and P were calculated using the equation:

$$ATTD\ (\%) = \frac{\left[Nutrient_i - Nutrient_f\right]}{Nutrient_i}\ x\ 100$$

where ATTD is the apparent total tract digestibility, $Nutrient_i$ is the total nutrient intake (g) from d 6 to 10; and $Nutrient_f$ is the total fecal output (g) of the nutrient originating from the diet fed from d 6 to 10.

Exp. 2. Growth Performance Study

Three hundred seventy-eight newly-weaned pigs (PIC L337×C24) initially weighing 6.6 ± 1.7 kg and 35 d of age were used in an 11-d growth assay. Pigs were blocked by initial weight and sex and randomly allotted to 1 of 3 dietary treatments using a randomized complete block design. Each treatment had 6 replications (pens) and 21 pigs per pen. Each pen contained a self-feeder and 2 nipple waterers to provide *ad libitum* access to feed and water. Pigs were housed in an environmentally controlled room in a tunnel-ventilated building located at Floridablanca, Pampanga.

A total of 3 experimental diets were formulated (Table 2). The first diet was a corn-soybean meal-based diet that served as the control. The next 2 diets were a corn-soy diet with 20% cassava meal without and with the inclusion of 200 mg/kg of a multi-enzyme product containing 6,250 visco unit/g of endo-1,4-ß-xylanase, 4,300 visco unit/g of endo-1,3(4)-ß-glucanase and 17 other enzyme activities, obtained from a fermentation broth of *Talaromyces versatilis* (Rovabio Advance P 25, Adisseo, France). The same batch of cassava meal was used in both experiments. All the diets were formulated with 3,402 kcal ME/kg and 1.37% SID Lys, and were prepared in meal form.

Pigs and feeders were weighed at d 40 (start of the experiment) and 51. At the conclusion of the experiment, data were summarized, and ADG, ADFI, and F:G were calculated for each treatment. Caloric efficiency was determined on an ME basis. Efficiencies were calculated by multiplying total intake by the energy level in the feed (kcal ME/kg) and dividing by total weight gain. Diarrhea score of each pen was assessed visually twice a day by two independent evaluators, with the score ranging from 1 to 5 (1 = normal feces, 2 = moist feces, 3 = mild diarrhea, 4 = severe diarrhea, and 5 = watery diarrhea). Data was summarized to overall diarrhea scores for each treatment. The average frequency of diarrhea (in days) was calculated by counting pig days with a diarrhea score of 3 or greater, and was also expressed as a percentage by dividing diarrhea days over total days in the experimental period x 100.

Data were analyzed as a randomized complete block design using the MIXED procedure of SAS (SAS Institute, Cary, NC) with pen as experimental unit. The model included diet as the fixed effect and block as the random effect. Least square means were calculated for each independent variable. When diet was a significant source of variation, least square means were separated using the PDIFF option of SAS. The α-level that was used to determine significance and tendencies between least square means were 0.05 and 0.10, respectively.

RESULTS AND DISCUSSION

On an as-fed basis, pigs fed the NSPase+Phytase diet tended (P<0.08) to have less DM intake compared with those fed the other treatments (Table 3). As a result, daily GE intake of pigs fed the NSPase+Phytase diet tended (P<0.09) to be less compared with the other treatments. However, there were no significant differences in fecal output and fecal and urinary GE loss among the treatments. Newly weaned pigs typically have lower feed intake as a result of multiple stresses imposed by weaning and are highly variable between individual pigs. This may explain the differences observed in DM intake among the treatments. The ATTD of GE tended (P=0.09) to be greater for the CM and NSPase diets compared with the CS diet. Furthermore, DE of the diet tended (P<0.06) to be greater with the

Table 2. Ingredient composition (as-fed basis) of experimental diets, Exp. 2[1].

Item	Dietary Treatment		
	Control	20% Cassava Meal	20% Cassava Meal + NSPase
Ingredients, %			
Corn, yellow	50.75	26.45	26.45
Cassava meal, 56% starch	--	20.00	20.00
Soybean meal	15.00	15.00	15.00
Whey permeate	11.77	11.77	11.77
Soybean meal, enzyme-treated	10.00	10.00	10.00
Soy protein concentrate	2.21	4.12	4.12
Hydrolyzed peptone	3.00	3.00	3.00
Coconut oil	2.44	4.88	4.88
L-lysine HCl	0.59	0.56	0.56
DL-methionine	0.24	0.28	0.28
L-threonine	0.18	0.19	0.19
L-tryptophan	0.08	0.07	0.07
L-valine	0.11	0.13	0.13
Monocalcium phosphate	0.94	0.94	0.94
Limestone	0.89	0.78	0.78
Salt	0.30	0.30	0.30
Zinc oxide	0.394	0.394	0.394
Vitamin-mineral premix[2]	0.13	0.13	0.13
Choline chloride 60%	0.35	0.35	0.35
Tiamulin 10% premix	0.035	0.035	0.035
CTC 15% premix	0.267	0.267	0.267
Acidifier	0.20	0.20	0.20
Toxin binder	0.05	0.05	0.05
Antimold	0.05	0.05	0.05
Antioxidant	0.012	0.012	0.012
Phytase	0.013	0.013	0.013
Cornstarch	--	0.02	--
Multi-enzyme	--	--	0.02
Total	100.000	100.000	100.000
Calculated Composition, %			
ME, kcal/kg	3,402	3,402	3,402
SID Lys	1.37	1.37	1.37

Table 2. Continued...

Item	Dietary Treatment		
	Control	20% Cassava Meal	20% Cassava Meal + NSPase
Analyzed Composition, %			
DM	89.59	90.62	90.56
CP (N × 6.25)	18.50	18.37	18.30
Crude fiber	2.06	2.30	2.36
Crude fat	4.20	4.48	4.45
Ash	5.69	5.90	5.87

[1]Dietary treatments: Corn-soy diet with no cassava meal and multi-enzyme (Control); Corn-soy diet + 20% cassava meal; and Corn-soy diet + 20% cassava meal supplemented with 200 mg/kg of a multi-enzyme product containing 6,250 visco unit/g of endo-1,4-ß-xylanase, 4,300 visco unit/g of endo-1,3(4)-ß-glucanase and 17 other enzyme activities, obtained from a fermentation broth of *Talaromyces versatilis* (NSPase; Rovabio Advance P 25, Adisseo, France).

[2]Provided the following quantities of vitamins and micro minerals per kg of complete diet: Vitamin A, 11,128 IU; vitamin D3, 2,204 IU; vitamin E, 66 IU; vitamin K, 1.42 mg; thiamin, 0.24 mg; riboflavin, 6.58 mg; pyridoxine, 0.24 mg; vitamin B12, 0.03 mg; D-pantothenic acid, 23.5 mg; niacin, 44 mg; folic acid, 1.58 mg; biotin, 0.44 mg; Cu, 10 mg as copper sulfate; Fe, 125 mg as iron sulfate; I, 1.26 mg as potassium iodate; Mn, 60 mg as manganese sulfate; Se, 0.3 mg as sodium selenite; and Zn, 100 mg as zinc oxide.

CM diet compared with the CS diet. However, ME did not significantly differ among the dietary treatments.

If NSP-degrading enzymes hydrolyze the NSP fractions in the diet, their anti-nutritional effects may be reversed and energy utilization should be improved. Indeed, previous studies have demonstrated the effectiveness of both mono-component (Li *et al*., 1996; van der Meulen *et al*., 2001) and multi-component (Kiarie *et al*., 2007; Ao *et al*., 2010) NSP-degrading enzymes in hydrolyzing NSP and increasing dietary energy utilization. However, the results of the present study indicate that supplementation with either of the two multi-enzyme combinations to diets with cassava meal did not improve energy digestibility. This was in agreement with the previous study of Iji *et al*. (2011) in broilers, where supplementation with NSP-degrading enzymes to diets containing either cassava chips or cassava pellets also failed to improve energy digestibility.

On average, the DE and ME of cassava meal (DM basis) determined in the present experiment were 3,606 and 3,496 kcal/kg, respectively (Table 3). The ME values obtained in the present experiment were within the range of previously published values (3,394 to 3,752 kcal/kg DM: Sauvant *et al*., 2004; NRC, 2012; Heuzé *et al*., 2016) for cassava meal. Compared to cereal grains, ME of cassava meal is less than both yellow corn (3,854 kcal/kg DM; NRC, 2012) and wheat (3,659 kcal/kg DM; NRC, 2012). Although cassava starch is more digestible than yellow corn in relation to its starch properties, corn still has higher ME due to its higher fat content.

Results of the present experiment indicate that supplementation with either of the two multi-enzyme combinations did not improve the DE and ME of cassava meal. To the best of our knowledge, there has been no previous study conducted to evaluate the effect of different multi-enzyme combinations on the energy value of cassava meal fed to nursery

Table 3. Daily energy balance (as-fed basis) of nursery pigs fed corn-soy diets with cassava meal (CM) supplemented with different multi-enzyme combinations (Exp. 1)[1,2].

Item	Corn-Soy Basal Diet	Basal + 40% Cassava Meal			SEM	*P*-Value
		No Enzyme	Multi-enzyme 1	Multi-enzyme 2		
			NSPase	NSPase + Phytase		
DM intake, g	328	360	356	228	0.04	0.08
GE intake, kcal	995	1,102	1,084	708	112	0.09
Fecal output, g	94	87	84	62	9	0.10
Fecal GE loss, kcal	102	93	86	67	9	0.11
Urinary output, g	1,678	1,392	1,504	1,264	7	0.85
Urinary GE loss, kcal	31	26	33	26	7	0.85
ATTD,[3] % of GE	89.42	91.47	92.11	90.34	0.75	0.09
DE of diet, kcal/kg	3,139	3,235	3,234	3,204	26	0.06
ME of diet, kcal/kg	3,016	3,146	3,116	3,064	44	0.20
DE of CM, kcal/kg	--	3,155	3,155	3,078	27	0.22
ME of CM, kcal/kg	--	3,128	3,053	2,922	100	0.97
DE of CM, kcal/kg DM	--	3,636	3,635	3,547	99	0.96
ME of CM, kcal/kg DM	--	3,607	3,519	3,363	123	0.13

[1]Data are least square means of 5 observations per treatment.

[2]Multi-enzyme 1 contains 25,000 visco unit/g of endo-1,4-ß-xylanase, and 17,200 visco unit/g of endo-1,3(4)-ß-glucanase and 17 other enzyme activities, obtained from a fermentation broth of *Talaromyces versatilis* (NSPase; Rovabio Advance P, Adisseo, France) and multi-enzyme 2 contains 6,250 visco unit/g of endo-1,4-ß-xylanase, 4,300 visco unit/g of endo-1,3(4)-ß-glucanase, 2,500 FTU/g of 6-phytase and 17 other enzyme activities, obtained from a fermentation broth of *Talaromyces versatilis* and *Schizosaccharomyces pombe* (NSPase+Phytase; Rovabio Max Advance P, Adisseo, France).

[3]ATTD = apparent total tract digestibility

pigs. Although cassava meal is a good source of carbohydrates, higher inclusion (40%) of cassava meal may affect energy digestibility and other nutrients because of its high fiber content. Bhuiyan and Iji (2015) also observed that ME intake was reduced in diets containing cassava chips or pellets, which may be due to the greater fiber content of the two diets when compared to yellow corn. The lack of a positive response from both multi-enzymes suggests that at the doses tested, there was no appreciable effect on energy utilization that can be measured even if there was hydrolysis of NSP in cassava meal. It may also be proposed that NSP in cassava meal may be complexed with other structural carbohydrates that are refractory to the effect of the multi-enzyme. Future research may focus more on identifying these potential interactions that may result in improved digestion of structural carbohydrates in cassava meal.

There were no significant differences in the ATTD of DM, OM, crude fat, and ash among the treatments; however, the CM diet had lower (P<0.01) ATTD of CP compared with the CS diet (Table 4). The NSPase diet had greater (P<0.01) ATTD of CP compared with the CS diet, but this effect was not observed with the NSPase+Phytase diet. This result conforms to the findings of Yin *et al*. (2010) where supplementation with NSP-degrading

Table 4. Apparent total tract digestibility (ATTD) of nutrients and dietary fiber in corn-soy diets with cassava meal supplemented with different multi-enzyme combinations fed to nursery pigs[1,2].

Item	Corn-Soy Basal Diet	Basal + 40% Cassava Meal			SEM	*P*-Value
		No Enzyme	Multi-enzyme 1	Multi-enzyme 2		
			NSPase	NSPase + Phytase		
ATTD[3], %						
DM	91.68	92.59	92.89	91.89	0.69	0.54
OM	93.19	94.19	94.28	93.42	4.21	0.98
Ash	76.53	74.89	77.76	73.32	2.36	0.54
CP	89.88[a]	82.19[b]	87.13[a]	83.93[b]	1.51	0.01
Crude fat	74.70	68.72	76.26	66.86	3.82	0.24
NDF	86.04[a]	75.90[b]	78.68[b]	77.30[b]	1.77	0.01
ADF	79.68[a]	66.41[b]	67.05[b]	66.92[b]	2.62	0.01
Ca	74.19[b]	84.35[a]	86.39[a]	84.57[a]	2.56	0.02
P	75.72	78.88	80.37	77.46	2.28	0.45

[1]Data are least square means of 5 observations per treatment.

[2]Multi-enzyme 1 contains 25,000 visco unit/g of endo-1,4-ß-xylanase, and 17,200 visco unit/g of endo-1,3(4)-ß-glucanase and 17 other enzyme activities, obtained from a fermentation broth of *Talaromyces versatilis* (NSPase; Rovabio Advance P, Adisseo, France) and multi-enzyme 2 contains 6,250 visco unit/g of endo-1,4-ß-xylanase, 4,300 visco unit/g of endo-1,3(4)-ß-glucanase, 2,500 FTU/g of 6-phytase and 17 other enzyme activities, obtained from a fermentation broth of *Talaromyces versatilis* and *Schizosaccharomyces pombe* (NSPase+Phytase; Rovabio Max Advance P, Adisseo, France).

[3]ATTD = apparent total tract digestibility

[a,b]Values within a row lacking a common superscript letter are different ($P \leq 0.05$).

enzymes improved the digestibility of CP and most AA. Meng and Slominski (2005) suggested that hydrolysis of encapsulating cell walls by multi-enzymes can disrupt the cell matrix, which may result in the release of structural proteins. The hydrolysis of NSP backbone may also increase enzyme access to the AA originally encapsulated by the carbohydrate complex.

The ATTD of NDF and ADF decreased (P<0.01) when cassava meal was added to the CS diet. However, supplementation of the multi-enzyme combinations to the CM diet did not affect ATTD of NDF and ADF. Although, there were many studies (Iji *et al*., 2011) that reported positive effects of enzyme supplementation on NSP digestibility; there are also numerous studies particularly in swine that have shown no effects from enzyme use. Enzymes are substrate-specific and the availability of the substrates affects the rate of reactions. In the present study, the major enzyme activities of the carbohydrases used were xylanases and ß-glucanases. The NSP in cassava meal is primarily insoluble NSP (5.4% vs. 0.8% soluble NSP), which is composed of cellulose, arabinose, and xylose.

The ATTD of Ca was greater (P<0.01) in the diets with cassava meal compared with the CS diet. However, supplementation of both multi-enzymes to the CM diet did not affect Ca digestibility. This suggests that Ca digestibility in cassava meal may be greater than both corn and soybean meal; however, this requires validation. There were no significant differences in the ATTD of P among the treatments. In the present study, ATTD of P in the CS diet was greater (75.7%) than other reported values (36.4%; Rojas and Stein, 2016) which may be due to relatively low proportion of P bound to phytate in these ingredients. This may possibly help explain the lack of an effect from the NSPase+Phytase diet on P digestibility.

There were no significant differences observed in ADG, ADFI, F/G, final BW and caloric efficiency among the treatments (Table 5). This indicates that including cassava meal up to 20% of nursery pig diets may be used without negatively affecting growth performance. However, multi-enzyme supplementation did not positively affect growth performance and caloric efficiency of pigs fed the diets with cassava meal. These results validate the findings in the energy balance study, where addition of the same multi-enzyme did not improve energy digestibility of the corn-soy diet with high inclusions of cassava meal. Numerous studies have also failed to observe any positive effect of multi-enzyme combinations on nursery growth performance (Grandhi, 2001; Olukosi *et al*., 2007).

In general, there was low incidence of diarrhea during the entire duration of the experiment. However, average diarrhea scores of nursery pigs fed the diet with cassava meal were lower (P<0.02) than those fed the control diet (Table 5). This indicates that the addition of cassava meal into nursery pig diets has no negative impact on diarrhea incidence, and in fact, may have improved fecal consistency. This is contrary to what was previously perceived by others in the use of cassava meal.

Therefore, it can be concluded that supplementation of diets containing cassava meal with the multi-enzyme combinations tested in this study do not improve energy value and nursery growth performance. Future research can focus on identifying potential interactions between structural and non-structural carbohydrates in cassava meal that may hinder effective enzymatic digestion to be able to develop better strategies in improving the feeding value of cassava meal.

Table 5. Effect of cassava meal and multi-enzyme supplementation on growth performance, caloric efficiency and diarrhea incidence in nursery pigs (Exp. 2)[1,2].

Item	Dietary Treatment			SEM	P-Value
	Control	20% Cassava Meal	20% Cassava Meal + NSPase		
Growth Performance					
Initial wt (d 40), kg	8.59	8.61	8.60	0.95	0.99
ADG, g	345	310	327	23.83	0.58
ADFI, g	478	459	456	30.95	0.86
F/G	1.39	1.48	1.41	0.07	0.62
Final wt (d 51), kg	14.12	13.76	13.82	1.34	0.98
ME efficiency, kcal ME/kg BW gain	4,729	5,035	4,797	238	0.64
Diarrhea Incidence					
Diarrhea score[3]	2.02[a]	1.72[b]	1.86[b]	0.07	0.02
Diarrhea days[4]	0	0	0	--	--
Frequency, %[5]	0	0	0	--	--

[1]Data are least square means with 6 replicates per treatment and 21 pigs per pen.
[2]Dietary treatments: Corn-soy diet with no cassava meal and multi-enzyme (Control); Corn-soy diet + 20% cassava meal; and Corn-soy diet + 20% cassava meal supplemented with 200 mg/kg of a multi-enzyme product containing 6,250 visco unit/g of endo-1,4-ß-xylanase, 4,300 visco unit/g of endo-1,3(4)-ß-glucanase and 17 other enzyme activities, obtained from a fermentation broth of *Talaromyces versatilis* (NSPase; Rovabio Advance P 25, Adisseo, France).
[3]Diarrhea score: 1 = normal feces; 2 = moist feces; 3 = mild diarrhea; 4 = severe diarrhea and 5 = watery diarrhea.
[4]Diarrhea days = number of pig days with diarrhea score ≥3.
[5]Frequency (frequency of diarrhea during the entire experimental period) = (diarrhea days/11 days) × 100
[a,b]Values within a row lacking a common superscript letter are different ($P \leq 0.05$).

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