β-GALACTOMANNANASE SUPPLEMENTATION IMPROVES PROTEIN DIGESTIBILITY AND GROWTH PERFORMANCE IN GROWING PIGS FED DIETS CONTAINING HIGH LEVELS OF COPRA MEAL

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ABSTRACT

The objective of this study was to determine the effects β-galactomannanase supplementation of of diets containing high levels of copra meal on nutrient digestibility and growth performance of growing pigs. A total of 120 pigs (Landrace X Large White X Duroc crossbreds; initial BW: 49 kg) were randomly allotted to 4 treatments following a 2 x 2 factorial arrangement in a completely randomized design. The factors were level of copra meal (20 and 25%) and β -galactomannanase supplementation. A commercial enzyme preparation containing β -galactomannanase with a minimum activity of 1000 units/g was added at the recommended inclusion rate of 5 g/kg of copra meal incorporated in the diet. Two weeks after the start of the experiment, 4 pigs from each treatment were randomly selected and were used in the digestibility trial. There were no copra meal level × enzyme interaction observed for all the parameters measured in the study. Apparent total tract digestibility (% ATTD) of DM, crude fiber, ether extract, and GE were not affected by copra meal level and β -galactomannanase supplementation. However, increasing copra meal level in the diet reduced (P<0.05) the ATTD of CP. The addition of β -galactomannanase to the diet increased (P<0.05) ATTD of CP. Increasing the copra meal level in the diet did not affect growth performance; however, ADG and F:G of growing pigs were improved (P<0.05) by β galactomannanase supplementation. In conclusion, the inclusion of copra meal can be raised up to 25% in growing pig diets, provided that diets are supplemented with β -galactomannanase.

Keywords: β-galactomannanase, copra meal, growth performance, nonstarch polysaccharides, nutrient digestibility

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INTRODUCTION

Despite the wide availability of copra meal in the Philippines, its inclusion in swine diets is limited due to high levels of non-starch polysaccharides (NSP). Non-starch polysaccharides are indigestible fractions of structural carbohydrates in plant ingredients which have negative effects on the digestibility of nutrients and consequently on growth performance of monogastrics. Mannans are the major form of NSP present in copra meal that can be classified in four sub-families: linear mannan, glucomannan, galactomannan, and galactoglucomannan (Petkowicz *et al.*, 2001 as cited by Moreira and Filho, 2008). Among these sub-families, galactomannans compose 61% of the total polysaccharides in the mature coconut kernel (Balasubramaniam, 1976). Galactomannan is composed of mannose and galactose units. The mannose elements form a linear chain consisting of $(1\rightarrow 4)$ - β -D-mannopyranosyl residues, with $(1\rightarrow 6)$ linked α -D-galactopyranosyl residues as side chain at varying distances, depending on the plant of origin (Sharma *et al.*, 2008).

Complete enzymatic hydrolysis of galactomannan to oligomers of galactose and mannose requires the action of β -mannanase, α -galactosidase and β -mannosidase (Ademark *et al.*, 1998; Chang *et al.*, 2006). The three enzymes are also involved in galactomannan degradation (Mujer *et al.*, 1984; Mendoza *et al.*, 1985; Ramirez, 1986). Majority of the studies have evaluated β -mannanase in improving the feeding value of copra meal (Teves *et al.*, 1992; Romano, 1999; Atinyao, 2000; Mercado, 2003; Khanongnuch *et al.*, 2006; Jang *et al.*, 2008). However, there are limited studies regarding the use of β -galactomannanase. In vitro studies have shown that β -galactomannanase effectively degrades galactomannanas in copra meal (Yokomizo, 2002); however, there are no in vivo studies evaluating the efficacy of β -galactomannanase in improving the feeding value of copra meal fed to pigs.

Therefore, this study was conducted to determine the effects of β -galactomannanase supplementation on nutrient digestibility and growth performance of growing pigs fed diets containing high levels of copra meal.

MATERIALS AND METHODS

Animals and Treatments

A total of 120 growing pigs (Landrace X Large White X Duroc crossbreds; initial BW of 49 kg) were used in a 35-d growth assay. Pigs were randomly allotted to 4 treatment groups following a 2 x 2 factorial arrangement in a completely randomized design. Factors were the level of copra meal (20 and 25%) and β -galactomannanase supplementation. Each treatment was replicated 3 times with 10 pigs per replicate pen.

Table 1.	Ingredient composition and calculated nutrient composition (as fed
	basis) of experimental diets.

	Treatment			
Item	20% Copra Meal		25% Copra Meal	
	without	with	without	with
	enzyme	enzyme	enzyme	enzyme
Ingredient, %				
Yellow corn	47.67	47.67	45.07	45.07
Soybean meal, US 46%	16.50	16.50	14.10	14.10
Copra meal	20.00	20.00	25.00	25.00
Rice bran, D1	7.00	7.00	7.00	7.00
Molasses	5.00	5.00	5.00	5.00
Coconut oil	0.80	0.80	0.80	0.80
MDCP	0.80	0.80	0.80	0.80
Limestone, fine	1.00	1.00	1.00	1.00
Salt	0.40	0.40	0.40	0.40
Choline chloride, 50%	0.10	0.10	0.10	0.10
L-Lysine	0.40	0.40	0.40	0.40
DL-Methionine	0.10	0.10	0.10	0.10
L-Threonine	0.00	0.00	0.00	0.00
Vitamin premix	0.03	0.03	0.03	0.03
Mineral premix	0.10	0.10	0.10	0.10
Mold Inhibitor	0.10	0.10	0.10	0.10
β-galactomannanase	-	+	-	+
TOTAL	100.00	100.00	100.00	100.00
Calculated Analysis, %				
CP (N × 6.25)	16.59	16.59	16.28	16.28
ME, kcal/kg	3,102	3,102	3,093	3,093
Lysine	0.91	0.91	0.86	0.86
Met + Cys	0.56	0.56	0.53	0.53
Threonine	0.63	0.63	0.60	0.60
Crude fat	5.43	5.43	5.82	5.82
Crude fiber	4.73	4.73	5.06	5.06
Calcium	0.70	0.70	0.70	0.70
Total P	0.63	0.63	0.64	0.64
Available P	0.27	0.27	0.26	0.26

Experimental Diets

A total 4 experimental diets were formulated (Table 1). A commercial enzyme preparation containing β -galactomannanase with a minimum activity of

1000 units/g was added at the recommended inclusion rate of 5 g per kg of copra meal incorporated in the diet. Animals were fed ad libitum with their corresponding diets and water was made available at all times.

Data Collection

Pigs were weighed at d 0 (start of the experiment) and 35 and total feed consumption were recorded to calculate for ADG, ADFI, and F:G. Two weeks after the start of the experiment, 4 pigs from each treatment were randomly selected and were used in the digestibility trial. Each pig was placed in an individual pen provided with a nipple drinker and feeder. Pigs were given their corresponding diets with 0.2% chromic oxide to serve as an inert marker. Grab samples of fresh feces were then collected from each pig for a total of 4 d after a 3 d adaptation period. After the digestibility trial, pigs were returned to their original pens.

Chemical Analyses

Diet samples were analyzed for proximate analyses, Ca, P, GE and chromic oxide concentration. For fecal samples, DM, CP, crude fiber, ether extract, GE, and chromic oxide concentrations were determined. All analyses were based on standard AOAC procedures (1995). Apparent total tract digestibility (% ATTD) of DM, CP, crude fat, crude fiber, and GE were calculated.

Economic Analysis

Gross margin analysis was employed with feed and pig cost as variable costs. Costs incurred due to the supplementation of the enzyme were included in the feed cost. Average income over feed and pig cost (IOFPC) was determined for each treatment. Average feed and pig cost was subtracted from average income to calculate for IOFPC.

Statistical Analysis

Data were analyzed using ANOVA following a 2 x 2 factorial design. Pairwise comparisons of treatment means were performed using the Duncan's Multiple Range Test. Statistical significance was set at $P \le 0.05$ for all statistical tests.

RESULTS AND DISCUSSION

Analyzed Composition of the Diets

On average, the CP, crude fiber, Ca, and total P concentration in the diets were 16.6, 4.61, 0.66, and 0.66%, respectively, which were close to the formulated values. However, the average ether extract concentration of the diets was 3.43%, which was less than the formulated value for crude fat (5.62%). This may be due to the wide variability in the residual oil content of copra meal. The average hemicellulose content of the diets was 8.25%.

Item	Diets with 20% Copra Meal	Diets with 25% Copra Meal	
Analyzed composition, %			
DM	87.22	87.01	
CP (N × 6.25)	16.94	16.16	
Ether extract	4.25	2.58	
Crude fiber	5.23	4.53	
Ash	6.40	5.39	
Calcium	0.76	0.55	
Total P	0.74	0.57	

Table 2. Analyzed nutrient composition (as-fed basis) of experimental diets.

Nutrient Digestibility

There were no copra meal level × enzyme interaction observed; therefore, only main effects were discussed (Table 3). No differences were observed for ATTD of DM, EE, CF and GE between pigs fed varying levels of copra meal. Likewise, there were no differences in the ATTD of DM, EE, CF and GE between pigs fed diets supplemented with or without β -galactomannanase.

Table 3. Main effects of diets with varying levels of copra meal supplemented with or without β-galactomannanase on the apparent total tract digestibility (ATTD, %) of DM, CP, ether extract (EE), crude fiber (CF), and GE in growing pigs^{1,2}.

	Level of Copra Meal		β-galactomannanase Supplementation		CV (%)
Item	20%	25%	without	with	(70)
ATTD of DM, %	75.2	74.6	74.2	75.6	3.4
ATTD of CP, %	63.9 ^a	59.4 ^b	59.5 ^b	63.8 ^a	5.2
ATTD of EE, %	36.8	26.7	26.1	37.4	41.9
ATTD of CF, %	36.4	31.6	33.3	34.7	23.9
ATTD of GE, %	71.0	70.0	69.5	71.6	4.0

¹Total of 120 growing pigs (Landrace × Large White × Duroc crossbreds; initial BW: 49 kg) with 10 pigs per replicate pen and 3 replicates per treatment.

49 kg) with to pigs per replicate per and 5 replicates per treat

 2 No copra meal level × enzyme interaction.

^{a,b}Within a row, means without a common superscript differ (*P*<0.05).

However, greater levels of copra meal in the diet reduced (*P*<0.05) ATTD of CP. This may be attributed to the low digestibility of protein in copra meal compared with the protein in soybean meal. Creswell and Brooks (1971) reported that protein digestibility in copra meal was 50.7%, which is less than 85.7% measured in soybean meal. However, the addition of β-galactomannanase in the diet improved (*P*<0.05) ATTD of CP. This implies that β-galactomannanase hydrolyzes the β bonds in β-galactomannan, thus breaking down the mannan backbone. Mannans serve as the main storage form of sugars in the endosperm of coconuts. Knudsen (1997) found that the endosperm cell wall encloses intracellular protein and lipid thus, preventing their digestion and absorption.

Growth Performance

There was no interaction observed between copra meal level and enzyme supplementation. Increasing the copra meal level in the diet from 20 to 25% did not affect growth performance; however, ADG and F:G of growing pigs were improved (*P*<0.05) by β-galactomannanase supplementation. This may be due to the observed improvement in ATTD of CP when growing pig diets were supplemented with β -galactomannanase. Though not all statistically significant, pigs fed the diets supplemented with β-galactomannanase also had numerically greater ATTD of DM, EE, CF and GE. The small but general enhancement in nutrient digestibility may explain the significant improvement in feed efficiency, which can be due to the ability of β-galactomannanase to counteract the negative effects of NSP on nutrient absorption.

	Level of Copra Meal		β-galactomannanase Supplementation		CV
Item	20%	25%	without	with	(%)
d 0 to 35					
ADG, kg	0.79	0.74	0.72 ^b	0.82 ^a	7.93
ADFI, kg	2.36	2.15	2.23	2.28	9.32
F:G	2.99	2.92	3.12 ^b	2.80 ^a	7.39

Table 4. Main effects of diets with varying levels of copra meal supplemented with or without β -galactomannanase on growth performance of growing pigs^{1,2}.

¹Total of 120 growing pigs (Landrace × Large White × Duroc crossbreds; initial BW: 49 kg) with 10 pigs per replicate pen and 3 replicates per treatment.

²No copra meal level × enzyme interaction.

^{a,b}Within a row, means without a common superscript differ (*P*<0.05).

Diet Economics

Average sales were greatest in pigs fed the diet with 20% copra meal supplemented with β -galactomannanase (Table 5). This was due to greater average selling weight of pigs in this treatment. In general, increasing the level of copra meal in the diet reduced average sales whereas β -galactomannanase supplementation increased average sales. In contrast, β -

galactomannanase supplementation increased feed cost whereas greater inclusion of copra meal in the diet reduced feed cost. Highest income over feed and pig cost was attained from sales of pigs fed the diet with 20% copra meal supplemented with β -galactomannanase. The added cost due to enzyme supplementation was compensated by the added income gained from higher selling weight of pigs. In general, β -galactomannanase supplementation had a positive effect on IOFPC.

Table 5. Diet economics.

	Diets with 20% Copra Meal		Diets with 25% Copra Meal	
	without	with	without	with
Item	enzyme	enzyme	enzyme	enzyme
Average Sales ¹ , PhP	6,261	6,678	6,117	6,497
Feed Cost ² , PhP	1,129	1,148	974	1,039
Pig Cost ¹ , PhP	4,271	4,174	4,056	4,154
Feed and Pig Cost, PhP	5,400	5,322	5,031	5,193
IOFPC, PhP	861	1,356	1,087	1,304

¹85 PhP per kg live weight.

²Cost of enzyme was incorporated in feed cost.

CONCLUSIONS

Based on the results of this study, it can be concluded that the inclusion of copra meal can be raised up to 25% in growing pig diets, provided that diets are supplemented with β -galactomannanase. It can be deduced that the advantage of using β -galactomannanase possibly lies in its capacity to release intracellular nutrients such as proteins that are bound and trapped by the NSP.

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REFERENCES

- Ademark P, Varga A, Medve J, Harjunpaa V, Drakenberg T, Tjerneld F and Stalbrand H. 1998. Softwood hemicellulose-degrading enzymes from *Aspergillus niger*: purification and properties of a β-mannanase. *J Biotechnol* 63: 199-210.
- AOAC. 1995. Official methods of analysis of AOAC International. 16th ed. Association of Official Analytical Chemists. Arlington, Virginia.
- Atinyao MB. 2000. Growth and physiologic responses of pigs and albino rats to copra meal diets supplemented with NSP-degrading enzyme. [Ph.D. Dissertation] College, Laguna, Philippines: University of the Philippines.
- Balasubramaniam K. 1976. Polysaccharides of the kernel of maturing and matured coconuts. *J Food Sci* 41: 1370-1373.
- Chang YT, Chen YS, Lin KT, Lin IF, Chen YF, Yen YH, Liu BL, and Chang YN. 2006. Characteristics of galactomannanase for degrading konjac gel. *J Mol Cat B: Enzymatic* 43: 153-157.
- Creswell DC and Brooks CC. 1971. Composition, apparent digestibility and energy evaluation of coconut oil and coconut meal. *J Anim Sci* 33: 366-369.
- Jang YD, Yun JH, Kim DH and Lee JH. 2008. Effects of mannanase addition to copra meal and palm kernel meal diets on growth performance and nutrient digestibility of growing-finishing pigs. *J Anim Sci* 86: 3.
- Khanongnuch C, Sa-Nguansook C and Lumyong S. 2006. Nutritive quality of β-mannanase treated copra meal in broiler diets and effectiveness on some fecal bacteria. *Int J Poultry Sci* 5: 1087-1091.
- Knudsen EB. 1997. Carbohydrate and lignin contents of plant materials used in animal feeding. *Anim Feed Sci Tech* 67: 319-338.
- Mendoza EMT, Samonte JL, Mujer CV, Dela Cruz NB, and Ramirez DA. 1985. Galactomannan metabolism in developing normal and makapuno coconut endosperm. *Trans Nat Acad Sci Tech (Phils.)* 7: 237-247.
- Mercado DG. 2003. Mannanase supplementation of swine diets with copra meal. [MS Thesis] College, Laguna, University of the Philippines Los Baños.
- Moreira, LRS and Filho EXF. 2008. An overview of mannan structure and mannan-degrading enzyme systems. *Appl Microbiol Biotechnol* 79: 165–178.
- Mujer CV, Ramirez DA, and Mendoza EMT. 1984. Alpha-D-galactosidase deficiency in coconut endosperm: its possible pleotropic effects in makapuno. *Phytochem* 23: 893-894.
- Ramirez DA. 1986. Genetics of makapuno: a genetic tumor of the coconut endosperm. *Trans Nat Acad Sci Tech (Phils.)* July 7-8, 1986, PICC, Manila.

- Romano NS. 1999. β-mannanase supplementation of swine diets with different levels of copra meal (*Cocos nucifera Linn.*). [MS Thesis] College, Laguna, University of the Philippines Los Baños.
- Sharma BR, Dhuldhoya NC, Merchant SU, and Merchant UC. 2008. A glimpse of galactomannans. *Science Tech Entrepreneur*. February 2008 Issue. Accessed 17 March 2010. lucidgroup.com/knowledge-center/glimpses-of-galactomannans.pdf.
- Teves FG, Zamora AF, Calapardo ML, and Luis ES. 1992. Nutritional value of copra meal treated with bacterial mannanase in broiler diets. *Philipp J Coco Stud* 15: 26-31.
- Yokomizo F. 2002. Mannose-containing copra meal composition. US Patent 6376220. Accessed 17 March 2010. http://www.freepatentsonline.com.

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