β-MANNANASE ENZYME SUPPLEMENTATION OF GROWER-FINISHER PIG DIETS WITH COPRA MEAL

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The use of copra meal in pig diets is limited due to its high level of non-starch polysaccharides (NSP) particularly β-mannans, which act as anti-nutritional factor causing gut viscosity and consequently poor nutrient digestibility. Supplementation of β-mannanase enzyme to corn-soybean based diets has been found to improve the performance of growing pigs. This study investigated the effect of adding β-mannanase enzyme to grower-finisher pig diets with different levels of copra meal. Five hundred growing pigs (24.52 kg mean BW) were randomly allotted to eight (8) dietary treatments, in a two-stage feeding program namely grower (first 4 weeks), and finisher (succeeding 9 weeks). The first experimental diets in both grower (CM10) and finisher (CM20) stages containing 10 and 20 percent copra meal respectively were not supplemented with β-mannanase enzyme. The next experimental diets in the grower (CM10+MAN) and finisher (CM20+MAN) stages contain the same level of copra meal as in the first but were supplemented with 400 IU of β-mannanase per kg. A third set of experimental diets in the

grower (CM15+MAN) and finisher (CM25+MAN) stages had higher levels of copra meal at 15 and 25 percent respectively, and were also supplemented with 400 IU of β-mannanase per kg. The fourth and last set of experimental diets for the grower (CM10+ME100) and finisher (CM20+ME100) stages contained the same level of copra meal as the first but had additional 100 kcal/kg of metabolizable energy compared to the other experimental diets. Body weight gain, feed intake, and feed per gain in the grower and finisher stages as well as for the whole duration of the study were taken and computed. No significant difference in weight gain was found among treatments in all stages but overall, diets supplemented with β-mannanase enzyme had significantly lower feed intake ($P<0.05$) and consequently better feed conversion. This study demonstrates the possibility of reducing cost by adding β-mannanase enzyme to diets with high levels of copra meal.

KEYWORDS: copra meal, β-mannanase enzyme, feed intake

INTRODUCTION

THE PHILIPPINES IS the top producer of copra in the world at more than 2.7 million metric tons in 2010 alone (Corpuz, 2010). As the raw material in the extraction of coconut oil, copra's major by-product is copra meal, which can be used as a raw material in grower-finisher and breeder (adult) pig diets. However, the use of copra meal in pig diets is limited due to its high fiber content and the presence of high levels of the non-starch polysaccharide (NSP) called β-mannan, which acts as an anti-nutritional factor causing gut viscosity and consequently poor nutrient digestibility. The economic benefits of using higher levels of copra meal in pig diets can be huge for the Philippine swine industry when considering the cheap cost of copra meal in the Philippines.

A few research studies have shown that supplementation with exogenous β-mannanase enzyme has improved the growth performance of newly-weaned and growing-finishing pigs by releasing extra metabolizable energy from diets containing high levels of soybean meal, which contains moderate levels of β-mannan, the same NSP found at high levels in copra meal. No study has been done to determine if the same β-mannanase

enzyme can also work on pig diets with significant levels of copra meal as it did on soybean meal-based diets.

REVIEW OF LITERATURE

β-mannans are non-starch polysaccharides found in some plant cell wall either as glucomannans or galactomannans. The glucomannans are comprised of β(1-4)-linked glucose and mannose units, while galactomannans consists of a β(1-4)-linked mannan backbone substituted with single units of α(1-6)-linked galactose (Choct, 1997). On a dry matter basis, copra meal contains 25-30% β-mannan as both pure and galactomannan (Sundu, Kumar, & Dingle, 2006).

β-mannans have been found to be deleterious to pig performance, compromising weight gain and feed conversion (Rainbird, Low, & Zebrowska, 1984; Nunes & Malmlof, 1992). This deleterious effect of β-mannan is associated with the viscous nature of all NSP's, their physiological and morphological effects on the digestive tract and the interaction with the microflora of the gut. The mechanisms include altered intestinal transit time, modification of the intestinal mucosa, and changes in hormonal regulation due to a varied rate of nutrient absorption (Choct, 1997). It is the inability of pigs to digest the β(1-4) glycosidic bonds in β-mannans due to the lack of the specific enzyme β-mannanase which causes this.

One of the first studies done on the use of pure β-mannanase on pig diets was by Pettey, Carter, Senne, & Shriver in 2002. They worked on a pure β-mannanase enzyme product fermented from *Bacillus lentus* to find out its effect on diets for weanling and growing-finishing pigs. For growing-finishing pigs, they fed sixty pigs (22.5 kg BW) with either a corn-soybean meal based (negative) control diet, the control diet with 2% soybean oil added to increase metabolizable energy (ME) by 100 kcal/kg (positive control), and the control diet with additional 0.05% β-mannanase enzyme product. After feeding the pigs until around 109 kg BW, they observed that addition of β-mannanase increased ADG compared to both the negative control diet and the positive control diet (with added soybean oil). They also observed that pigs fed the positive control diets and β-mannanase had similar feed efficiency. This led them to suggest that β-mannanase may

provide the equivalent energy of 100 kilocalories per kilogram (kcal/kg) of feed.

It was only last year when a study to confirm the work of Pettey, et al. (2002) was done by Bass, Frank, Johnson, Maxwell, & Lee (2010) at the University of Arkansas, using a pure β-mannanase enzyme from *Bacillus* sp. WL-1. In their feeding trial, a total of 140 grower pigs (65 lb body weight) were fed a 3-phase corn-soybean based diet either with a negative control (NC), the NC diet supplemented with 200,000 IU of β-mannanase (MAN2), the NC diet supplemented with 400,000 IU of β-mannanase (MAN4), and with a positive control diet (PC) which is the NC diet containing additional 45.4 kcal/lb (100 kcal/kg) of metabolizable energy from tallow. Pigs fed the PC diets had a 4.1% higher overall average daily gain and 8.5 lb additional body weight at the end of the study compared to pigs fed the NC diet. Diets supplemented with β-mannanase (MAN2 & MAN4) had a lower feed intake in phase 1, phase 2, and over-all, and a lower feed per gain at each level of β-mannanase addition during phase 1 and overall. Although Bass et al. (2010) could not show that β-mannanase supplementation can improve grower-finisher pig performance similar to adding 100kcal/kg of energy in the diet, as shown by Pettey et al. (2002), they were able to show an improvement in energy utilization in pigs fed β-mannanase.

To date, no work has been done to confirm the studies of Pettey et al. (2002) and Bass et al. (2010) using grower-finisher pigs fed diets containing copra meal. For the interest of the swine raising and feedmilling industries in the Philippines where copra meal is a cheap and available feed raw material, a study is needed to find out if significant amounts of metabolizable energy can be extracted from β-mannanase supplemented pig grower and finisher diets containing copra meal.

MATERIALS AND METHODS

Animals

Five hundred cross-bred, growing pigs, with a mean weight of 24.52 kg, were randomly allotted to 20 equal groups, with each group randomly allotted to 20 pens of similar size. These pens were randomly allotted to four dietary treatments, each treatment

having five replicates. Each pen with 25 pigs was considered as one experimental unit.

Experimental Diets

Eight experimental diets were formulated and fed in two stages. The first four iso-nitrogenous diets were fed for the first four weeks, the grower stage; while the other four iso-nitrogenous diets were fed on the succeeding nine weeks, the finisher stage.

In the grower stage, the negative control diet, called CM10, is a grower diet containing 10% copra meal. The next experimental diet (CM10+MAN) is the same diet as CM10 but supplemented with 400 IU of β-mannanase per kg. The next experimental diet (CM15+MAN) is a grower diet containing 15% copra meal supplemented with 400 IU of β-mannanase (CTCZyme) per kg. The last diet, considered the positive control diet (CM10+ME100) is the same diet as CM10 but with the metabolizable energy (ME) content increased by 100 kcal/kg. Diets CM10, CM10+MAN, and CM15+MAN are all iso-caloric. A summary of the experimental diets in the grower stage can be seen in Table 1.

Table 1. **Summary of Experimental Diets in Grower Stage.**

Experimental Diet	Formulation
CM10 (Negative Control)	Grower Diet with 10% Copra Meal
CM10+MAN	Treatment 1 Diet + 400 IU β-mannanase
CM15+MAN	Grower Diet with 15% Copra Meal + 400 IU β-mannanase
CM10+ME100 (Positive Control)	Treatment 1 Diet + 100 kcal/kg of metabolizable energy

In the finisher stage, the negative control diet, called CM20 is a finisher diet containing 20% copra meal. The next experimental diet (CM20+MAN) is the same CM20 diet but supplemented with 400 IU of β-mannanase (CTCZyme) per kg. The next experimental diet (CM25+MAN) is a finisher diet containing 25% copra meal supplemented with 400 IU of β-mannanase (CTCZyme) per kg. The last diet, considered as the positive control diet (CM20+ME100), is the same CM20 diet but with the metabolizable energy (ME) content increased by 100 kcal/kg. CM20, CM20+MAN, and

CM25+MAN are all iso-caloric. A summary of the experimental diets in the finisher stage can be seen in Table 2.

Table 2. **Summary of Experimental Diets in Finisher Stage.**

Experimental Diet	Formulation
CM20 (Negative Control)	Finisher Diet with 20% Copra Meal
CM20+MAN	Treatment 1 Diet + 400 IU β-mannanase
CM25+MAN	Finisher Diet with 25% Copra Meal + 400 IU β-mannanase
CM20+ME100 (Positive Control)	Treatment 1 Diet + 100 kcal/kg of metabolizable energy

The β-mannanase enzyme product (CTCZyme) used comes from the fermentation of *Bacillus* sp. WL-1 and is the same enzyme used by Frank et al. in 2009. It was provided by CTCBIO Inc. of Seoul, Korea. All experimental diets did not contain any antibiotic as growth promotant or in-feed medication.

Data Collection

Initial and final body weights, and feed intake (consumption) per pen, during the grower and finisher stages as well as for the whole duration (overall) of the trial were recorded. Body weight gain per pen was computed based on the recorded initial and final body weights. Based on the feed consumption and weight gain data, the feed conversion ratio (feed per gain) of each pen was computed. The incidence, date and weight of all mortality if any were also recorded. Weight gain of pigs that died before the end of the feeding trial was included in the computation of FCR per pen.

Statistical Analysis

Treatment effect on body weight gain and feed per gain per pen was determined using Analysis of Variance (ANOVA). Differences among treatment means were considered significant at P values <0.05. When found significantly different, a t-test would be conducted to determine which particular treatment mean differed from the others ($P<0.05$).

Table 3. **Mean Performance of Pigs Fed the Four Dietary Treatments**[1]

Parameters	Dietary Treatments				P Value[2]
	CM10/CM20	CM10+MAN/ CM20+MAN	CM15+MAN/ CM25+MAN	CM10+ME100/ CM20+ME100	
Body weight gain per pen, kg					
Grower stage	515.80	504.40	517.72	524.70	0.565
Finisher stage	1,122.30	1,078.90	1,073.98	1,128.20	0.611
Overall	1,638.10	1,583.30	1,591.70	1,652.90	0.539
Feed intake per pen, kg					
Grower stage	1,362^{a}	1,266^{ab}	1,170^{b}	894^{c}	.001
Finisher stage	3,720^{a}	3,672^{ab}	3,552^{b}	2,082^{c}	5.26E-15
Overall	5,082^{a}	4,938^{b}	4,722^{c}	2,976^{d}	6.23E-13
Feed per gain					
Grower stage	2.64^{a}	2.52^{ab}	2.26^{b}	1.71^{c}	0.002
Finisher stage	3.33^{a}	3.42^{a}	3.31^{a}	1.85^{b}	5.4E-09
Overall	3.11^{ab}	3.12^{a}	2.97^{b}	1.81^{c}	1.08E-09

[1] A total of 500 grower pigs with a mean weight of 24.52 kg were used at the beginning of the 13-week study (25 pigs per pen, 5 pens per treatment, 4 treatments). Grower stage (first 4 weeks) used iso-nitrogenous diets among four treatments, and finisher stage (next 9 weeks) also used iso-nitrogenous diets among four treatments. Grower stage CM10 diet had 10% copra meal without β-mannanase. Grower stage CM10+MAN diet had 10% copra meal with 400 IU of β-mannanase. Grower stage CM15+MAN diet had 15% copra meal with 400 IU of β-mannanase. Grower stage CM10+ME100 diet had 10% copra meal but was 100 kcal/kg higher in metabolizable energy compared to the other treatments. Finisher stage CM20 diet had 20% copra meal without β-mannanase. Finisher stage CM20+MAN diet had 20% copra meal with 400 IU of β-mannanase. Finisher stage CM25+MAN diet had 25% copra meal with 400 IU of β-mannanase. Finisher stage CM20+ME100 diet had 20% copra meal but was 100 kcal/kg higher in metabolizable energy compared to the other treatments.

[2] Data were analyzed using ANOVA with 2 factors, where 1 factor is the pen or replicate (considered as blocking factor) and the other is the treatment. Means within a row without a common superscript letter are significantly different ($P<0.05$).

Time and Place of Study

Feeding trial for this study was done at Tecolu Farms in Calinan, Davao City from March to May 2011.

RESULTS

Table 3 shows the mean performance of pigs fed the four experimental diets and the corresponding P values from the ANOVA.

Grower Stage

At the end of the grower stage, all pigs had a mean body weight of 45.59 kg. Body weight gain during the grower stage was not significantly different among treatments although pigs on the positive control diet (CM10+ME100) had the highest body weight gain.

However, feed intake in the grower stage was significantly different among treatments ($P<0.05$) with pigs on the positive control diet (CM10+ME100) consuming significantly the least amount of feed. Pigs on the negative control diet (CM10) consumed the most followed by pigs on CM10+MAN although not significantly different. Pigs on CM15+MAN had the next lower feed intake consuming significantly lesser than pigs on CM10 but not significantly lesser than pigs on CM10+MAN.

As a result of the significantly different feed intake among treatments in pigs during the grower stage, feed per gain was likewise significantly different among treatments ($P<0.05$) with pigs on the positive control diet (CM10+ME100) giving the significantly lowest feed consumption per gain in weight of 1.71. As in feed intake, pigs on the negative control diet (CM10) had the highest feed per gain followed by pigs on CM10+MAN and then pigs on CM15+MAN. Feed per gain of pigs on CM10+MAN was not significantly lower than pigs on the negative control diet (CM10) and was not significantly higher than pigs on CM15+MAN, which had a significantly lower feed per gain than pigs on the negative control diet (CM10).

Finisher Stage

At the end of the finisher stage, all pigs had a mean body weight of 92.95 kg. As in the grower stage, body weight gain during the finisher stage was not significantly different among treatments with pigs on the positive control diet (CM20+ME100) having the highest body weight gain.

Similar to the grower stage, feed intake during the finisher stage was likewise significantly different among treatments ($P<0.05$) with pigs on the positive control diet (CM20+ME100) consuming the significantly least amount of feed. Pigs on the negative control diet (CM20) consumed the most followed by pigs on CM20+MAN although not significantly different. Pigs on CM25+MAN had the next lower feed intake consuming significantly lesser than pigs on the negative control diet (CM20) but not significantly lesser than CM20+MAN.

As in the grower stage, feed per gain during the finisher stage was likewise significantly different among treatments ($P<0.05$) with pigs on the positive control diet (CM20+ME100) giving the significantly lowest feed consumption per gain in weight of 1.85. However, feed per gain of pigs on the negative control diet (CM20), CM20+MAN and CM25+MAN did not differ significantly.

Overall

When combining both grower and finisher stages, overall body weight gain was not significantly different among treatments with pigs on the positive control diets (CM10+ME100/CM20+ME100) showing the highest body weight gain.

Overall feed intake was significantly different among treatments ($P<0.05$) with pigs on the positive control diets (CM10+ME100/CM20+ME100) consuming the significantly least amount of feed. Pigs on the negative control diets (CM10/CM20) had the significantly highest feed intake, followed significantly by pigs on CM10+MAN/CM20+MAN, and then followed significantly by pigs on CM15+MAN/CM25+MAN.

Due to the significant difference in overall feed intake, overall feed per gain was significantly different among treatments ($P<0.05$) with pigs on the positive control diets (CM10+ME100/CM20+ME100) giving the significantly lowest feed consumption

per gain in weight of 1.81. It is interesting to note however, that pigs on CM15+MAN/CM25+MAN had a significantly lower overall feed per gain compared to pigs on CM10+MAN/CM20+MAN. Although not significantly different, this is also true in both the grower and finisher stages.

DISCUSSION

Contrary to the findings of Pettey et al. (2002), body weight gain of pigs was not increased significantly when given β-mannanase supplemented diets or the positive control diets (with additional 100 kcal/kg of ME), although pigs given the positive control diets gave the numerically highest body weight gain in the grower and finisher stages as well as overall.

An increase in dietary energy concentration is usually associated with a reduction of voluntary feed intake (Noblet, 2006). The decreasing trend in feed intake observed among pigs from those consuming the negative control diets (CM10/CM20), followed respectively by β-mannanase supplemented diets with increasing copra meal level (CM10+MAN/CM20+MAN and CM15+MAN/CM25+MAN) could only be explained by an increasing dietary energy concentration. The increasing level of β-mannans available for degradation by β-mannanase enzyme in these diets could have been the source of additional mannose (monosaccharide) moieties available for energy use.

Bass et al. (2010) also observed this declining feed intake when they increased β-mannanase supplementation from 0 to 200,000 IU, and 400,000 IU per ton. In their study however, it was the level of supplementation that was increased, while the amount of substrate remained the same.

Pettey et al. (2002) described a similar decrease in feed intake among pigs when 100 kcal/kg of ME from soybean oil was added to the diet. Bass et al. (2010), on the other hand, did not observe this when 100 kcal/kg of ME from tallow was added. In this study the 100 kcal/kg ME added to the positive control diets came from coconut oil. The different properties of the oil/fat used in these three studies might explain why pigs performed differently in the positive control diets.

Tallow contains almost equal amounts of saturated (52.1%) and unsaturated (47.9) fatty acids, 88% of which are long-chain

(with more than or equal to 16 carbons). Soybean oil contains predominantly unsaturated (84.9%) fatty acids, 95.1% of which are long-chain (with more than or equal to 16 carbons) like tallow. Coconut oil, unlike tallow and soybean oil contains predominantly saturated (91.9%) fatty acids, 75.5% of which are medium-chain fatty acids (with 10 to 14 carbons) (NRC, 1998). Unlike long-chain fatty acids, medium-chain fatty acids supply a quick source of energy when supplied in the diet, because they have a smaller molecular size, and thus are hydrolyzed and absorbed faster in the intestinal mucosa (Back & Babayan, 1982). Medium-chain fatty acids leave the intestinal mucosa by the portal venous system whereas long-chain fatty acids follow the lymphatic system, thus medium-chain fatty acids reach the liver more rapidly. Furthermore in the liver, medium-chain fatty acids cross the double mitochondrial membrane very rapidly because unlike long-chain fatty acids, they do not require the presence of carnitine to cross the mitochondrial membrane. (Bremer, 1980). It is inside the mitochondria where fatty acids undergo β-oxidation to produce acetyl-Coa which subsequently enters the Krebs cycle to produce energy in the form of ATP.

The unique metabolic pathway taken by the medium-chain fatty acids in coconut oil could be the reason why Cera, Mahan, & Reinhart (1989) observed that coconut oil had the highest apparent fat digestibility compared to corn oil and tallow in pigs 4 weeks post-weaning. Pigs fed coconut oil also had a higher weight gain than those fed tallow or corn oil. Corn oil also contains predominantly long-chain fatty acids. Using newly weaned pigs (4.9 kg BW), Li, et al. (1990) compared coconut oil with soybean oil and several coconut and soybean oil combinations. They observed that from 0 to 35 days post weaning, pigs fed coconut oil alone had higher ADG and better feed efficiency.

The feed intake of pigs fed the positive control diets in this study cannot be compared to the feed intake of pigs fed the positive control diets in the study of Pettey et al. (2002) and Bass et al. (2010) because the type of oil used to provide the additional 100 kcal/kg of ME was different. Furthermore, the strategy of using coconut oil to provide additional energy to the positive control diet may have affected feed intake not as a result of the additional 100 kcal/kg ME but due to the unique metabolic pathway taken by its medium-chain fatty acids during digestion and metabolism. Future studies which seek to determine the effect of increasing

the energy concentration of feed should not use coconut oil as a source of additional energy.

IMPLICATIONS

This study has shown that β-mannanase enzyme supplementation of pig diets with high levels of copra meal reduces feed intake but is able to maintain growth rate, consequently improving feed conversion. β-mannanase enzyme supplementation therefore allows the possibility of using higher levels of copra meal in pig grower and finisher diets for the purpose of reducing feed cost while maintaining growth performance.

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