Effect of Enzyme Supplementation in Total Mixed Ration Containing Oil Palm Frond Silage on Productive Performance of Goat

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Thesis Title Effect of Enzyme Supplementation in Total Mixed Ration Containing Oil Palm Frond Silage on Productive Performance of Goat

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ABSTRACT

This experiment was conducted to study the effects of supplementing the TMR containing oil palm frond (OPF) silage with different levels of enzyme on feed intake, apparent digestibility, rumen ecology, growth performance and production cost of goat. Twenty four post-weaning Boer X Thai Native crossbred male goats with initial body weight (BW) of 11 to 18 kg, were arranged to receive four dietary treatments in a randomized complete block design. The diet used in the study contained 60% oil palm frond silage and 40% concentrate (DM basis). The enzyme mixture produced by *Aspergillus* spp. BCC 274 approximately contained 1 X 10^7, 9 X 10^6, 2 X 10^6, 1 X 10^6 and 2 X 10^6 unit/kg dry weight for xylanase, β-glucanase, cellulase, mannanase and amylase, respectively, were supplemented to the concentrate portion at 0, 2, 4 and 6 g/kgDM of the TMR. The experiment period of the study was 90 days. The results showed that the supplementation of enzyme to the TMR did not affect (P>0.05) intake of DM (55.64 to 57.04 g/kgBW$^{0.75}$), OM (48.41 to 50.68 g/kgBW$^{0.75}$), CP (9.12 to 9.25 g/kgBW$^{0.75}$) and ADF (15.71 to 16.56 g/kgBW$^{0.75}$), except NDF intake which was quadratic effected by enzyme

supplementation ($P<0.01$). Goats receiving TMR supplemented with enzyme at 2 g/kgDM had numerically highest average daily gain (ADG) and weight gain (40.86 g/d and 3.67 kg, respectively) and the best feed per gain (10.76). Digestibility coefficients of DM, OM and CP were not significantly affected by the enzyme supplementation. A quadratic effect of enzyme supplementation on NDF digestibility coefficient ($P<0.01$) was observed. Increasing level of enzyme supplementation in TMR resulted in a linear ($P<0.01$) and cubic ($P<0.01$) increase in ADF digestibility coefficient.

Regarding rumen fermentation parameters, ruminal fluid pH, overall means of total volatile fatty acid (VFAs), including the amount of acetic acid (C_2), propionic acid (C_3), and butyric acid (C_4) in rumen fluid, and blood urea nitrogen (BUN) concentration were not significantly different ($P>0.05$) among treatments. However, overall means of ruminal NH_3-N concentration was significantly lower in goat receiving TMR supplemented with enzyme at 2 g/kgDM (12.56 mg/dl) than that of goat receiving TMR with no enzyme supplementation (15.83 mg/dl). Overall means of bacteria population (1.14 to 1.55 $x10^{10}$ cell/ml) and fungi zoospores (4.74 to 5.45 $x10^{6}$ cell/ml) in the rumen fluid did not affect by the enzyme supplementation ($P>0.05$) except the population of protozoa. Overall means of protozoa population, including both *Holotrich* sp. and *Entodiniomorphs* sp. increased (linear: $P<0.05$) as a result of an increase in level of enzyme supplementation.

Considering production cost, rearing goats with TMR supplemented with enzyme at 0, 2, 4 and 6 g/kgDM had a profit during 90 days rearing, even cost of labor was included. The range of profit was 375.00 to 480.45 baht/head. However, when excluding the cost of labor, rearing goat with TMR supplemented with enzyme

had more profit which ranged from 566.25 to 671.70 baht/head. The rearing cost per kg weight gain was lowest when goat receiving TMR supplemented with enzyme at 2 g/kgDM (54.57 baht/head) followed by goat receiving TMR supplemented with enzyme at 0, 4 and 6 g/kgDM (64.34, 66.74 and 74.56 baht/head, respectively).

Based on this experiment, the application of enzyme at 2 g/kgDM in TMR containing OPF silage could increase ruminal availability of slowly digestible carbohydrate and improve goat performance. Furthermore, rearing goat with TMR supplemented with enzyme at 2 g/kgDM showed the highest profit and the cheapest cost per kg weight gain.

Keywords: enzyme, total mixed ration, oil palm frond, silage, goat

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List Abbreviations and Symbols

ADF	= acid detergent fiber
ADFI	= acid detergent fiber intake
ADL	= acid detergent lignin
AIA	= acid insoluble ash
BUN	= blood urea nitrogen
BW	= body weight
CF	= crude fiber
CP	= crude protein
DM	= dry matter
DMI	= dry matter intake
EE	= ether extract
NDF	= neutral detergent fiber
NDFI	= neutral detergent fiber intake
NFE	= nitrogen free extract
OM	= organic matter
OMI	= organic matter intake
PCV	= packed cell volume
SEM	= standard error of the mean
TDN	= total digestible nutrient
TMR	= total mixed ration

CHAPTER 1

Introduction

1.1 Introduction

Roughage is crucial factor as feed for ruminant. It is because of a unique digestive tract, complex stomach of the ruminants i.e., they have rumen for fermentative digestion by ruminal bacteria, protozoa, and fungi (Kamra, 2005). They capable to grow well just consuming forage or agricultural by-products which are consisted of high crude fiber content inedible food for human and mostly monogastric animals. So far, grass is commonly used as roughage source for ruminant but in the tropical area, the availability of grass is mostly depending on the season. In the rainy season the yield of grass is much higher than in the dry season. Then the utilization of grass in the dry season can be replaced by using agricultural by products.

It can be seen from the statistical data that South East Asia Region is important in palm oil production. The producers of palm oil in the world are dominated by the countries in this region such as Indonesia, Malaysia, and Thailand. According to USDA (2010), the world production of oil palm in 2008 was led by Indonesia 47%, Malaysia 39%, and Thailand 3.5%. Various by-products are produced from palm oil industry, for instance, oil palm frond (OPF), palm kernel cake, free fruit bunches, and palm oil mill effluent. OPF is one of the by-products that are abundantly produced which had a potential as a source of roughage for ruminant (Dahlan *et al*., 2000; Kawamoto *et al*., 2001). Abu Hassan *et al.* (1998) reported that OPF has been used to substitute tropical grass in Malaysia. However, the utilization of OPF as feed for ruminant is still limited due to their low digestibility which affected feed intake.

Many researches have been carried out relating to the use of OPF for animal feed to overcome the constraint as mentioned above such as OPF pellet, chopped OPF, OPF silage, and NaOH treated OPF (NaOH-OPF). Wan Zahari *et al.* (2008) showed that the digestibility and intake of NaOH-OPF was higher than other treatments (chopped, pelleted, and silage). However, NaOH is caustic and dangerous, so the safe treatment must be considered. Silage becomes one method of consideration in OPF treatment. Although, OPF silage intake is lower than NaOH treatment but for digestibility is comparable (Wan Zahari *et al.*, 2008). Applied OPF silage is more beneficial comparing with fresh chopped OPF in terms of handling, storage, minimized labor usage, and easier to distribute and also as one way for animal feed preservation.

Serving OPF silage together with concentrate in total mixed ration (TMR) is suggested to improve their palatability or intake. The reported optimal level of OPF in TMR on dry matter basis (DM) was 50% for beef cattle and 30% for dairy cattle and goat/sheep (Abu Hassan *et al.*, 1998). However, Roddoung *et al.* (2010) showed that Anglo-Nubian X Thai Native crossbred male goat fed TMR contained OPF silage:concentrate ratio of 50:50 had the highest average daily gain (ADG) but, no significant difference was found regarding feed intake and feed conversion ratio when compared with the goat fed TMR contained OPF silage and concentrate ratio of 60:40.

To improve feed digestibility by ruminant, exogenous enzyme supplementation has been recently used, but results are often inconsistent. Apparently the inconsistent results can be contributed to a number of factors including diet composition, type of enzyme preparation, component of enzyme activities and amount

of enzyme provided, enzyme stabilities, and method of application. Regarding the factors related to the diet, the effectiveness of enzyme has been shown to vary with forage (Wallace *et al.*, 2001; Colombatto *et al.*, 2003), enzyme levels and application methods (Wang *et al.*, 2001; Giraldo *et al.*, 2004), and the component of the diet to which the enzyme is added i.e., the forage component, the concentrate component or the complete TMR (Beauchemin *et al.*, 2003). Some positive effects of supplementing the diet with exogenous enzyme have been reported in dairy cows and beef steers, but the use of enzymes in the feeding of small ruminants has received little attention. Therefore, the hypothesis of this study is that the supplementation of enzyme could improve the nutritive values of TMR containing OPF silage and productive performance of goat.

1.2 Review of Literature

1.2.1 Characteristics of enzyme

Enzyme is a catalyst that makes a chemical reaction move faster. Mostly the component of enzyme is protein built by amino acids (Bohager, 2006). The enzyme reaction includes the formation, breakdown, and rearranging of molecules to provide organisms with the energy and materials needed to live and function.

The availability of enzyme in living organism is in “inactive” form. Consequently, there are certain conditions to support enzyme to work properly or become “active” form, such as pH, temperature, enzyme concentration, substrate

concentration, and the presence of inhibitors or activators (Wortingthon, 1972). The mechanism of enzyme activity is shown in Figure 1.

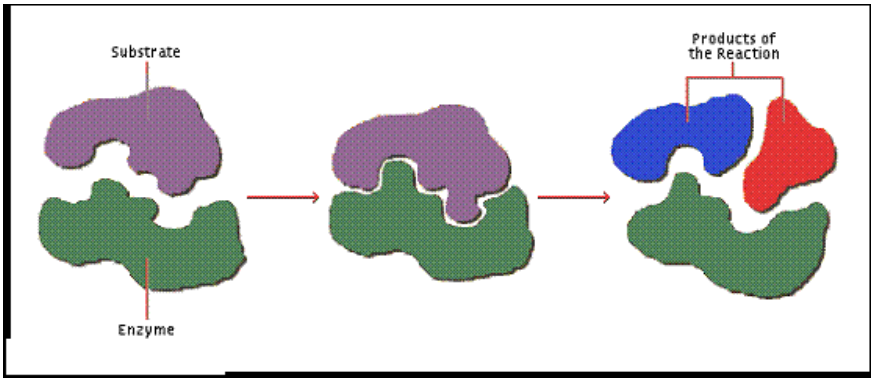

Figure 1. The mechanism of enzyme activity
Source: Brochez (2006)

Enzymes are classified by the type of chemical reaction catalyzed. According to NC-IUBMB (2010), the category of enzymes is oxidoreductases, transferases, hydrolases, lyases, isomerases, and ligases. Furthermore, the most enzymes that found are hydrolases. However, in digestive tract of animal, there are three basic categories; proteases, lipases, and amylases (also known as carbohydrases). Each of these categories has their own specific role, metabolically (creating energy in the body), and digestively (assisting with extracting energy from nutrients). Proteases hydrolyze proteins; lipases break down lipids (fats) and amylases break down carbohydrates. Nowadays, exogenous enzymes have been used extensively to remove anti-nutrition factors from feeds and to increase nutrient digestibility of monogastric animals. Many commercial enzymes, derived from bacterial (*Lactobacillus acidophilus*, *Lactobacillus plantarum*, and *Streptococcus faecium* spp.) and fungal (*Aspergillus* spp., *Tricoderma reesei*, *Penicillium* spp., and *Saccharomyces cerevisiae*) species (Rode *et al.*, 2001 cited from Pendleton, 1998),

contain relatively little actual fibrolytic enzyme i.e., cellulase, hemicellulase, and xylanase, activity (Kung *et al*., 1998). Furthermore, the optimal conditions for most commercial enzyme activity are a temperature of approximately 60ºC and pH between 4 and 5. Table 1. describes the activity of different commercial enzymes at different pH and temperature. The other comparison of cellulase enzyme activity at different pH is also shown in Figure 2.

Table 1. pH and temperature of cellulase activity profiles[1] of three commercial plant degrading enzyme product

	Product 1			Product 2			Product 3		
Temperature (ºC)	39	50	60	39	50	60	39	50	60
pH									
4.0	81.0	81.6	100	65.8	79.0	93.6	43.3	67.1	75.8
5.0	57.6	57.4	81.7	69.0	85.3	100	43.1	70.7	100
6.0	33.7	40.9	49.3	67.3	76.4	73.1	32.8	38.5	40.8
7.0	20.2	26.1	30.9	43.3	52.9	16.8	18.3	13.7	1.23

[1]Activity profiles determined using remazolbrilliant blue dyed carboxymethyl cellulose and expressed as a percentage of the maximum activity.
Source: Rode *et al.* (2001)

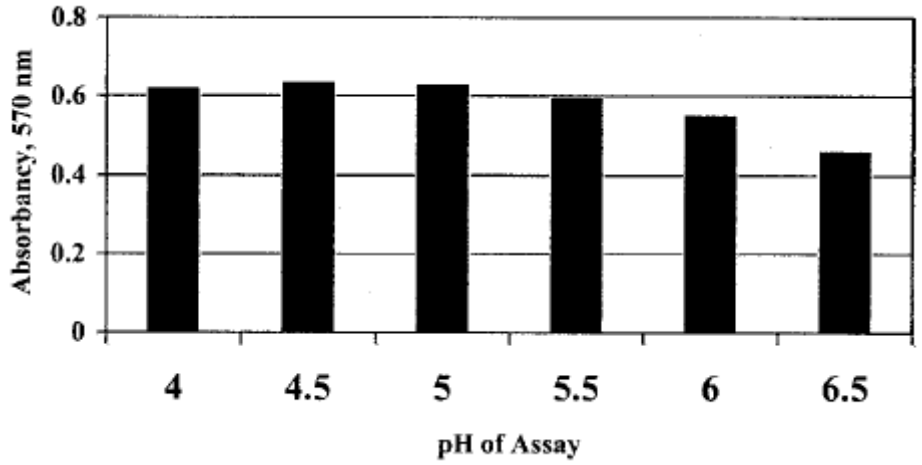

Figure 2. Enzyme activity of the cellulase D complex at different assays of pH
Source: Kung *et al.* (2002)

Both Table 1 and Figure 2 illustrate that pH and temperature play an important role on enzyme activity. It can be seen from Figure 2 that cellulase D has high activity in acid condition (pH 4 to 5). Its activity is different relative to xylanase B and C activity (Figure 3), the activity of xylanase C is higher at pH 6.5, but the activity of xylanase B is similar to that of cellulase D (acid condition).

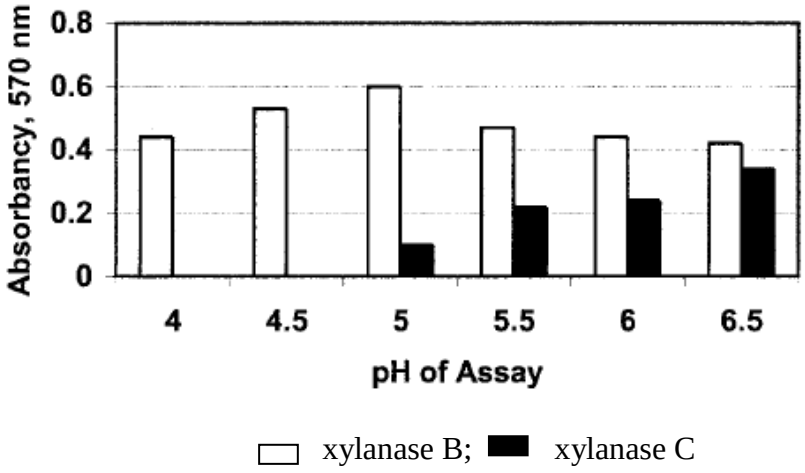

Figure 3. Activity of the xylanase B and C enzyme complexes at different assay pH. Source: Kung *et al.* (2002)

1.2.2 Enzyme for ruminant

Cellulose and hemicellulose are quantitatively the most important structural carbohydrates present in ruminant diets. Rumen micro-organisms produce enzyme that catalyze their hydrolysis, but the complex network formed by structural carbohydrates and lignin reduced their digestibility and restricted efficient utilization of feeds by ruminant. Many attempts have been made to overcome this limitation. The use of exogenous fibrolytic enzyme such as cellulase, hemicellulase and xylanase holds promise as a means of increasing forage utilization and improving the productive efficiency of ruminant. Beauchemin *et al.* (2003) cited from Pendleton

(2000) informed that enzyme products for ruminant diet came from fungi (mostly *Trichoderma longibrachiatum*, *Aspergillus niger*, *Aspergillus oryzae*) and bacteria (mostly *Bacillus* spp.). To date, commercial fibrolytic enzymes were originally developed for detergent, textile, pulp/paper, food, and monogastric feed industry (Rode *et al.*, 2001; Beauchemin *et al.*, 2003). Several enzyme products were originally developed as silage additive (Feng *et al.*, 1996). In addition to fiber-degrading enzyme, these products also have secondary enzyme activities, including amylase, protease, and pectinases.

Cellulases are specific for breaking down cellulose. But, there are many specific enzymes which are contributed to cellulase activity. The dominant enzyme contributing to cellulose hydrolysis are endocellulase, exocellulase, and β-glucosidase. Generally, endocellulase hydrolyzes the cellulose chains at random to produce cellulose oligomers of varying degrees of polymerization; exocellulase hydrolyzes the cellulose chains from nonreducing end, producing cellobiose and β-glucosidase hydrolyzes short-chains cellulose oligomers, and cellobiose to glucose (Beauchemin *et al.*, 2003).

The major enzyme involved in degrading the xylan to soluble sugars is xylanases which include endoxylanases and β-1,4-xylosidases. Other hemicellulase enzymes involved primarily in the digestion of side chains include β-mannosidase, α-L-arabino-furanosidase, α-D-glucoronidase, α-D-galactosidase, acetyl xylan esterases and ferulic acid esterase (Beauchemin *et al.*, 2003 cited from Bhat and Hazlewood, 2001).

Exogenous fibrolytic enzyme supplementation in ruminant has been utilized for improving feed utilization and animal performance, despite observed

responses have been highly variable. One of the factors that contribute to the inconsistency results is optimal conditions for enzyme activities. Whereas a temperature of approximately 60^{o}C and pH between 4 and 5 are the optimal conditions for most commercial enzymes, normal ruminal conditions are the temperature of 39^{o}C and pH closer to 6.7. Others inconsistent results can be also contributed to a number of factors including diet composition, type of enzyme preparation, amount of enzyme provided, enzyme stabilities, methods of enzyme application and the level of animal productivity. Regarding the factors related to the diet, the effectiveness of enzyme has been shown to vary with forage, enzyme levels and application method, and the component of the diet to which the enzyme is added i.e., the forage component, the concentrate component or TMR. In the last decade, researchers have reexamined the potential use of exogenous enzymes for ruminants due to higher feed costs, lower costs of enzyme production, and the availability of more active and better defined preparation.

There are many commercial fibrolytic enzymes for ruminant and each contains different composition. For example Natugrain 33-L, an enzyme that developed for poultry consists of at least 6,000 endo-1,3 (4)- β-gluconase unit per gram and 2,750 endo-1,4- β-xylanase unit per gram (Beauchemin *et al.*, 2000). The other enzymes containing cellulase and xylanase are products from Alltech Inc. (Reddish and Kung, 2007). In addition, Krause *et al.* (1998) explained about Pro-Mote®, which mainly contained cellulase and xylanase and low level residual of amylase activity.

1.2.3 Use of exogenous enzyme in ruminant

Exogenous enzymes have been used extensively to remove anti-nutritional factors from feed and to improve the nutritive values of feeds for non-ruminants but they are not routinely used in the diets of ruminants. Several enzyme products evaluated as feed additives in ruminant diets were originally developed as silage additive (Feng *et al.*, 1996). In recent years, there has been interest in the potential use of enzymes in ruminant diets. This interest stems from the high cost of livestock production, the availability of new enzyme mixtures and the potential economic returns realized with effective enzyme supplements. Several studies showed the use of feed enzymes substantially improved feed digestibility and animal performance although some studies reported no effects and even negative responses. Modyanov and Zelner (1983) cited by McAllister *et al.* (1999) explained that the inconsistencies may have arisen from differences in diet composition, enzyme application method, activity and stability of enzyme preparations, and even the level of animal productivity (Beauchemin *et al.*, 2003).

1.2.3.1 Level of enzyme supplementation to ruminant

The optimum level of enzyme supplementation in ruminant diet is still in process of investigation due to the variable results. Different researchers have different suggestion about the optimum level. Sometimes, the addition of enzyme did not show significant effect on several measured variables, but the others showed significant effect on the feed intake, digestibility and production of ruminant (meat or milk).

The research conducted by Kung *et al.* (2000), offered forage (60% corn silage and 40% lucerne hay; dry matter (DM) basis) treated with increasing levels (0, 1, 2.5 ml/kg of TMR) of an enzyme product (FinnFeeds Int, containing mainly cellulase and xylanase activity) to dairy cow, suggested that high levels of enzyme treatment might not be beneficial. In addition, Beauchemin *et al.* (2000), used an enzyme (Natugrain 33-L) supplement containing relatively high concentration of β-glucanase, xylanase and endocellulase at 0, 1.22 or 3.67 l/tonne of TMR, reported that a low concentration of enzyme supplementation improved dry matter intake (DMI) and digestibility of dairy cow, whereas a high concentration of enzyme supplementation increased intake, but not digestibility. Boonthep *et al.* (2010), supplemented enzyme produced by *Aspergillus* spp. BCC 274 (containing 1 X 10^7, 9 X 10^6, 2 X 10^6 , 1 X 10^6 and 2 X 10^6 unit/kg dry weight for xylanase, β-glucanase, cellulose, mannanase and amylase, respectively) to TMR (60% OPF silage and 40% concentrate; DM basis), reported that the digestible organic matter (DOM) and metabolizable energy (ME) of TMR supplemented with enzyme at 6 g/kgDM were significantly ($P<0.05$) lower than that of TMR supplemented with enzyme at 2 g/kgDM. These studies clearly demonstrate that the response to level of enzyme supplementation is not linear. High levels of enzyme addition can be less effective than low levels, and the optimal level of enzyme supplementation may depend on diet.

Besides positive effect, no significance effects of enzyme supplementation in ruminant diet were also obtained. Reddish and Kung (2007) studied the addition of enzymes mixture contained xylanase and cellulase to TMR (26% corn silage, 17% alfalfa silage, 7% chopped alfalfa hay, and 50% concentrate;

DM basis) at 10 g/cow/day. They reported that the enzyme supplemented did not enhance feed nutrition or change milk composition of lactating cow. The enzyme mixture also had no effect on the in vitro digestion of TMR even when added in high dose (12.5 g/l of ruminal and buffer fluid). Furthermore, when added to a diet for lambs (4 g/d), nutrient digestion was unaffected.

Table 2 summarizes the responses regarding the level of enzyme supplementation to ruminant.

Table 2. Animal responses to various levels of enzyme supplementation

No.	Name/producer of enzyme	Mainly enzyme	Level of enzyme	The result	Source
1.	FinnFeeds Int,	cellulase and xylanase	0, 1, 2.5 ml/kg of TMR	High level of enzyme might not be beneficial.	Kung *et al.* (2000)
2.	Natugrain 33-L	β-glucanase, xylanase, and endocellulase	0, 1.22 or 3.67 l/tonne of TMR	-low level increased DMI and digestibility -high level only increased DMI	Beauchemin *et al.* (2000)
3.	BIOTEC	xylanase β-glucanase, cellulase, mannanase, and amylase	0, 2, 4, 6 g/kgDM	-ME of TMR supplemented with enzyme at 6 g/kgDM were significantly ($P<0.05$) lower than that of TMR supplemented with enzyme at 2 g/kgDM.	Boonthep *et al.* (2010)
4.	Alltech, Inc	xylanase and cellulase	10 g/cow/day	-no effect on feed nutrition or milk composition.	Reddish and Kung (2007)

1.2.3.2 Method of enzyme application to ruminant

There are various methods of providing enzyme to ruminant diet. For instance, the enzyme is applied in a liquid form by spraying to the diets, mixed to concentrate, mixed to roughage, mixed to TMR, and directly delivered to the rumen. According to Kung *et al.* (2002), the applying enzyme (cellulase D and sultanase B or cellulase D and xylanase C) in liquid form gave positive effect on milk production in lactating cow. Enzymes were mixed, diluted with water and sprayed (within 30 min of mixing) onto corn silage and hay (10 L/tonne of fresh forage). Then the pelleted

concentrate was mixed with the forage to form TMR that was fed to animals within 30 min of enzyme treatment. However, Yang *et al.* (1999) applied an enzyme product contained primarily cellulase and xylanase activity, to dry forage or to both dry forage and concentrate, reported that no differences between both of methods on feed digestion and milk production of dairy cow. The other researchers have found that adding enzyme directly to concentrate to be more effective (Beauchemin *et al.*, 1997; Rode *et al.*, 1999; Yang *et al.*, 2000). In addition, Sutton *et al.* (2003) conducted a research to investigate effect of three different methods of applying enzyme in TMR for dairy cow i.e., sprayed on the TMR 1 h before feeding (TMR-E), sprayed only on the concentrate the day before feeding (Conc-E) or infused into the rumen for 14 h/d (Rumen-E). The enzyme used (Biovance Technology, Inc.) was extracted from *Trichoderma longibrachiatum* and contained xylanase and endoglucanase activities of 26,483 and 2645 mol/min/g. The dose of enzyme used in each treatment was 1.64 l/1000 kg. Within this experiment, feed intake and milk yield were highest on TMR-E. Total tract digestibility was also highest on TMR-E for dry matter, organic matter, and starch. The result of Sutton *et al.* (2003) suggested that applying enzyme to the TMR is recommended. However, Yang *et al.* (2000) reported increased milk production and digestibility of the diet when enzymes contained relatively high xylanase and low cellulase activities, in liquid form were added to the concentrate portion of a dairy cow diet, but not when they were added directly to TMR. Bowman *et al.* (2002) examined the effects of adding an enzyme product (Promote N.E.T Agribrands International, St Louis, Mo) containing primarily xylanase and cellulase, to various portion of a TMR i.e., to the concentrate portion (45% of the TMR), to the supplement (4% of TMR), and to the premixed (0.2% of TMR). Digestibility of

organic matter (OM), neutral detergent fiber (NDF) and acid detergent fiber (ADF) in total tract was increased in comparison with the control (no enzyme added) when enzymes were added to the entire concentrate. Enzyme treatments that were applied to a smaller portion of the diet showed only numerical increase in digestibility over the control. Cows receiving the enzyme product to the concentrate had numerically higher fat corrected milk compared to the control cows. These results suggested that the proportion of the diet which the enzyme is applied must be maximized to ensure a beneficial response. Adding enzyme to a small portion of the diet may allow rapid passage of enzyme from the rumen that lessens the enzyme effect in the rumen.

1.2.4 Ruminant response to enzyme supplementation

The application of fibrolytic enzyme supplementation was done because of some positive responses. Supplementing the diet with fibrolytic enzyme have been reported in dairy cows and beef steers, but the use of enzymes in the feeding of small ruminants has received little attention.

1.2.4.1 Beef cattle response to enzyme supplementation

Several studies have conducted to examine enzyme supplementation in growing cattle or beef cattle diet (Krause *et al.*, 1998; McAllister *et al.*, 1999; Wang *et al.*, 2004). The results of those researches revealed that adding enzyme increased the digestibility and also improved the animal performance, although the response of animal was inconsistent (McAllister *et al.*, 1999). Beauchemin *et al.* (2003) reported that the variation of the animal response might depend on the physiological status of animal and the condition of the experiment. McAllister *et al.* (1999) studied the effect

of supplemental fibrolytic enzyme applied to TMR on growth performance of steers. In this study, treating the silage portion of an 82.5% barley silage with a commercial cellulase and xylanase enzyme (Finfeeds International Ltd., Malborough, UK) at 0, 1.25, 3.5 or 5.0 L/tonne DM tended to linearly increase (P=0.08) final weights of steer. ADG, feed intake, and feed efficiency tended to be quadratic (P=0.06, P=0.04 and P=0.03, respectively) related to these enzyme concentration from days 0 to 56, but not overall (days 0 to 120). The conditions under a consistent positive response in animal performance from enzyme supplementation remained to be defined.

The results of adding enzymes to high grain diets have been more consistent than those for high forage diet. Applying an enzyme product (Xylanase B, Biovance Technologies Inc., Omaha, NE) to a 95% barley-based diet improved feed efficiency by 6% (Beauchemin *et al.*, 1997). Similarly, Krause *et al.* (1998) reported a 28% increase in ADF digestibility using enzyme mixture (Pro-More, Biovance Technologies Inc, Omaha, NE) contained mainly cellulase and xylanase activity. In addition, acetate to propionate ratio tended to decrease which suggested that enzymes might have increased ruminal starch digestion as a result of enhanced digestion of barley hulls. Furthermore, Lewis *et al.* (1996) declared that application of fibrolytic enzyme (Grazzyme®, FinnFeeds International, Marlborough, Wiltshire, U.K) containing cellulase, xylanase, cellobiase, and glucose oxidase activity, to barley increase the availability of fermentable carbohydrate. Ruminal concentrations of volatile fatty acid (VFA) were greater (P<0.10) at 16 h for steers fed enzyme treatments (average 136.5 mmol/l) than for control (104.0 mmol/l). Concentrations of VFA were greater (P<0.01) in steer when enzyme was applied to barley than when

enzyme was applied to forage, which might be an effect of improved fermentation of barley rather than grass hay structure.

1.2.4.2 Dairy cattle response to enzyme supplementation

There are many studies discussing about enzyme supplementation on digestibility and production in dairy cattle. Measurements of total tract digestibility in dairy cows have generally shown positive responses to enzymes with variable but often significant increases in the digestion of DM, OM, NDF, and ADF. Beauchemin *et al.* (2000) reported increased DM and OM intake in dairy cows fed a TMR supplemented with a low or high concentration of enzyme containing mainly β–glucanase, xylanase, and endocellulase activity. But total tract digestibility only increased with the low concentration of enzyme. As a result, intake of digestible nutrients was increased to a greater extent for cows fed the low concentration of enzyme than for cows fed the high concentration of enzyme. Yang *et al.* (2000) applied an enzyme product (Biovance Technologies Inc., Omaha, NE) with relatively high xylanase and low cellulase activities to the TMR (E-TMR) or to the barley-based concentrate portion (E-Conc) and observed a higher total tract DM digestibility for cow fed E-Conc than for cow fed the control diet (no enzyme) and intermediate for cows fed E-TMR. Although, applying an enzyme to the TMR did not significantly increase the digestibility of DM, exogenous enzymes added either to TMR or to concentrate increased digestibility of OM and protein compared to the control diet.

An increase in milk production has been reported in some studies when dairy cow diets were supplemented with enzymes but not in others. Kung *et al.* (2000) showed that treating a diet in which forage was based on corn silage and alfalfa hay

with enzymes (cellulase and xylanase) improved milk production with no mark effects on milk composition. Cows fed forage treated with a cellulase enzyme (3,700 carboxymethyl cellulase units/kg forage DM) produced 2.5 kg more milk than cows fed the control. In addition, Yang *et al.* (2000) reported that exogenous enzymes applied to the concentrate portion of the diet of cows in early lactation had a potential to increase milk production due to enhanced nutrient digestibility in the total tract. Applying enzymes to the TMR before feeding improved digestibility, but had no effect on milk production. This results is consistent with the results of Bowman *et al.* (2002) who reported that cows in mid lactation fed a diet to which an enzyme product characterized by xylanase and cellulase activities (Promote N.E.T. Agribrands International, St. Louis, Mo) was applied to a concentrate (45% of TMR) numerically increased milk production. On the other hand, Reddish and Kung (2007) used the enzyme mixture contained xylanase and cellulase activities over abroad range of pH (4 to 7) to TMR for lactating cow and observed no effect on milk production or milk composition. The results are interpreted to indicate that exogenous enzymes should be applied to a substantial portion of the diet to ensure their effectiveness.

1.2.4.3 Sheep/Goat response to enzyme supplementation

Compared to cattle, the research on the use of enzyme in the feeding of sheep or goat is limited. Reddish and Kung (2007) reported that mixed enzyme (xylanase and cellulase) did not enhance nutrient digestibility of lambs. Moreover, Giraldo *et al.* (2008) directly delivered enzyme contained endoglucanase and xylanase activities (Fibrozyme, Alltech Inc., Nicholasville, KY, 12 g/d) to rumen of sheep fed a mixed grass hay:concentrate (70:30; DM basis) diet and observed no effects on diet

digestibility, urinary excretion of purine derivatives, ruminal pH or concentrations of ammonia nitrogen (NH_3-N), and total VFA. In contrast, molar proportion of propionate were greater (P=0.001) and acetate:propionate ratio was lower (P<0.001) in enzyme supplemented sheep. In addition, enzyme supplementation tended to increase (P=0.06) number of cellulolytic bacteria at 4 h after feeding. The results indicated that supplementing enzyme directly into the rumen increased the fibrolytic activity in ruminal fluid without a prefeeding feed-enzyme interaction. The research conducted by Titi and Lubbadeh (2004) regarding cellulase enzyme derived from *Trichoderma* group supplementation on productive responses of pregnant and lactating Awassi ewes and Shami goat revealed no significant effects on feed intake nor birth weight, but enzyme supplementation increased the weaning weight, milk production, and improved milk composition. Both milk yields of Awassi ewes and Shami goat increased from 45.76 kg to 50.21 kg and from 54.49 kg to 61.23 kg, respectively. The improvement of milk composition was indicated by increasing of total solids. The amount of total solids from treated ewes and goat (18.33 and 13.40%, respectively) was higher than untreated (control) group (16.91 and 12.53%, respectively). Increasing total solids indicated increasing amount of milk fat and protein content in milk of treated ewes while no effect was observed on milk of treated goat. The results suggested that improvement of milk production without apparent change in feed intake was through improved feed utilization.

1.2.5. Total Mixed Ration

The common feeding systems for ruminant are cut and carry system and grazing. Usually for conventional ruminant raising, combination between both of

those systems is adopted, grazing in the morning to afternoon, and cut and carry system during evening to night. Andrade-Montemayor *et al.* (2005) reported that traditional feeding system separated between forage and concentrate. Moreover, this feeding method, gives negative effect such as fluctuation of ruminal pH. Consequently, it will disturb growth of rumen microorganism, limit use of feed and cause digestion problem (Abijaoudé *et al.*, 2000).

TMR consists of forage and concentrate mixture, avoiding animal selection. Then, the utilization of agricultural by-products can be optimized. The benefit of TMR feeding system is reported by Amaral-Philips *et al.* (2002), such as, increasing of milk production in dairy cattle, decreasing of feed cost, improvement of feed intake and cow health, and improvement of animal reproductive performance. The improvement of feed intake using TMR can minimize animal selection and sometimes it is an easy method to introduce new feedstuff having low palatability but high nutritive values. TMR is also good for feeding the goat that has bad habit in selection of what they eat. In addition, TMR feeding provides continuity of substrate for ruminal microorganism, so supply of nitrogen and carbohydrate is balanced and it is good for microbial protein synthesis (Colin-Schoellen *et al.*, 2000).

Andrade-Montemayor *et al.* (2005) conducted a research which compared between TMR and conventional rations (CR) and found that no significant effect on DOM intake, although the grain from CR is fermented faster than grain in TMR. Generally, TMR feeding contributes to a low cost because feedstuff that has high nutritive values but low palatability can be used.

1.2.6 Oil palm frond silage

Oil palm (*Elaeis guineensis* Jacq.) is originated from West Africa and originally spreaded to the other countries along the river and then cleared by human to cultivate (Hartley, 1977). According to Kartesz (2010), oil palm is classified as shown below:

Kingdom : *Plantae* – Plants
Subkingdom : *Tracheobionta* – Vascular plants
Superdivision : *Spermatophyta* – Seed plants
Division : *Magnoliophyta* – Flowering plants
Class : *Liliopsida* – Monocotyledons
Subclass : *Arecidae*
Order : *Arecales*
Family : *Arecaceae* – Palm family
Genus : *Elaeis* Jacq. – oil palm
Species : *Elaeis guineensis* Jacq. – African oil palm

OPF is the most abundant by-product produced from oil palm plantation and had a potential as a source of roughage for ruminant (Dahlan *et al*., 2000; Kawamoto *et al*., 2001). It was taken from annual pruning with the production of OPF around 82.5 kg/palm/yr (Chan *et al.*, 1980). An OPF is made up of three components i.e. petiole, rachis and leaflets (Figure 4). About 70% of the DM in the OPF is from the petiole and the rest from leaves and rachis (Wan Zahari *et al.*, 2004). The leaves contain a higher percentage of crude protein (CP), ether extract (EE), ash, and nitrogen free extract (NFE) than the total frond (Aim-oeb *et al.*, 2008). The chemical composition of fresh OPF are 45.8%, 7.17%, 93.3%, 6.7% and 52.47% of DM, CP, OM, ash and ADF, respectively (Dahlan *et al.*, 2000). Thus, OPF has a great potential as a roughage source for ruminant. Wan Zahari *et al.* (2004) reported that in

some oil palm factory in Malaysia, all of OPF from pruning process is sent to chop and continued to pelleting process or cubing. The pelleted OPF is given for ruminant, in this case for beef cattle. Then for cubed OPF is for goat, sheep, and dairy cattle. As well as pelleted or cubed, preservation of OPF can be done by ensiling.

Figure 4.The oil palm tree and the component of oil palm frond

A number of processing techniques have been developed to improve the feeding qualities of OPF i.e., preservation as silage, alkali treatment, pelletizing with urea, and molasses treatment. It can be revealed from Table 3 regarding the comparison of chemical composition of processed OPF. In addition, Kawamoto *et al.* (2001) reported that even though NaOH treated OPF had highest digestibility among the other processed, OPF silage was more palatable. The pelleted OPF is the most palatable product but has the lowest digestibility. Perhaps, it is because of their particle size which is faster escaping from rumen. Shorter retention time in rumen

decreases the digestibility of OPF pelleted. On the other hand, according to information from Table 3, the losses of nutritive values of OPF silage are not too much. Therefore, OPF silage is highly recommended as suitable method to preserve OPF. Many studies suggested that good quality silage could be produced without using any additives (Ishida and Abu Hassan, 1992; Dahlan *et al.*, 2000; Aim-oeb *et al.*, 2008; Rattanagoson, 2009).

Table 3. The comparison of nutritive values (% DM) of fresh OPF, OPF silage, and the other treatments of OPF

Items	Fresh OPF[1)]	OPF silage[2)]	OPF pelleted[2)]	NaOH-OPF[2)]
DM	-	30.2	87.5	28.1
CP	4.7	4.7	4.3	4.8
CF	38.5	38.5	-	-
NDF	78.7	80.4	81.1	73.3
ADF	55.6	-	-	-
EE	2.1	1.7	1.8	1.7
Ash	3.2	4.9	5.5	10.2

[1)] Abu Hassan *et al.* (1998)
[2)] Kawamoto *et al.* (2001)

1.2.7 Goat production in Southern Thailand

Goat is one of small ruminant that is familiar for Southeast Asia society. According to Cronje (1998) and Devendra (1999) for such developing countries by keeping of small ruminant plays as cash income from sales of their products and a safety net of capital assets to face risks and misfortune in harsh environments. In addition, the ownership of those kinds of animals, in the rural area, shows the prestige and wealthy symbol. So, belonging that animal is not only because of economic reason but also has social impact for people surroundings. In Thailand,

raising goats have main purposes for fulfilling meat demand (Supakorn and Pralomkarn, 2009) and milk production.

According to Bouwman (1997) in FAO report, the population of sheep and goat in developing countries including China in 1990 to 2010 was going to increase fast around 1.3% per year. Then, the last reported by FAO (2003) the population of goat in Thailand in 2002 was around 150,000 heads. It was higher than sheep population, which was only 42,700 heads. DLD (2008) cited by Wattanachant (2008) reported that the population of goat in Thailand increased during 1998-2007. The last data, in 2007 there were 444,774 heads of goat in Thailand (Table 4) which concentrated in the southern region that especially meet Thai Muslim's demand in meat (Wattanachant, 2008).

Table 4. Statistics of goat in Thailand during 1998 to 2007

Years	Regions				Total
	Central	Northeast	North	South	
	----------head----------				
1998	15,314	1,537	10,607	103,446	130,904
1999	16,070	1,573	13,588	101,614	132,845
2000	19,000	2,635	17,419	105,173	144,227
2001	37,789	12,295	24,134	114,279	188,497
2002	37,356	4,573	29,579	106,436	177,944
2003	52,967	5,021	43,410	112,519	213,917
2004	62,950	12,354	39,729	135,043	250,076
2005	109,681	13,974	55,310	159,390	338,335
2006	111,742	15,014	56,149	141,245	324,150
2007	162,926	21,423	86,373	174,052	444,774

Source: Adapted from DLD (2008) cited by Wattanachant (2008)

In Southern Thailand, several breeds of goat are raised, including Thai Native (TN), Anglo-Nubian (AN), Alpine, Saanen, Toggenburg and crossbred among them (Saithanoo *et al.*, 1991). The characteristics of TN are similar to Indonesian or

Malaysian native goat, kambing kacang. They have light weight around 22 to 23 kg in average for mature goat (Saithanoo and Milton, 1988), the color is brown, white, black or the combination, having erect ear, and triangular head (Saithanoo *et al.*, 1991). Moreover, the appearance of crossbred is to increase the performance of goat. Generally, TN performance is lower than breed from European. Kochapakdee *et al.* (1995) reported that cross breeding of TN goat with European breed can improve the live weight and growth rate. The increase of growth rate is because of increasing feed intake and feed conversion efficiency, as inheritance from European breeds.

Nowadays, Boer's breed was imported for upgrading TN performance (Wattanachant, 2008). There are several advantages of choosing Boer's for cross breeding to improve native breed performance such as good carcass, fast weight gain, easy care, high fertility, good mothering, excellent for weeds control through grazing, and good quality of meat (Lu, 2010). According to Thongchumroon *et al.* (2002) cited by Wattanachant (2008), crossbred between TN and Boer performed the highest live weight and growth, compared to AN crossbred or the other breeds.

Within the review of literature, exogenous enzyme supplementation could improve feed intake, feed digestibility, milk yield, and milk composition in ruminant, despite observed responses are highly variable. Regarding the amount of enzyme supplementation, the response is not linear. High levels of enzyme addition can be less effective than low levels, and the optimal level of enzyme supplementation may depend on diet. The proportion of the diet which the enzyme is applied must be also maximized to ensure a beneficial response. The supplementation of enzyme to the roughage source from agricultural by-products becomes the reasonable solution for

increasing the by-products utilization. OPF, by-products from oil palm plantation that produced abundantly in Southern Thailand, has limitation of low digestibility and low intake. Therefore, the supplementation of enzyme to OPF based diet is needed to increase the nutritive values as well as production performance of ruminant. Goat is one of the important small ruminants which rising in Southern Thailand to fulfill meat demand. The supplementation of enzyme to OPF based diet is expected to increase goat production in the region.

The outcome that will be achieved from the study is a basic information for applying enzyme supplementation in TMR as goat feed for maximizing goat productivity while minimizing cost of production. Then, the farmer can adopt this technology to increase their income. The data from this research is, moreover, becoming a reference for ruminant feeding research in the future.

1.3 Objectives

1. To determine the effect of enzyme supplementation in TMR containing OPF silage on feed intake, daily gain and feed per gain of goat.
2. To determine the effect of enzyme supplementation in TMR containing OPF silage on apparent digestibility and rumen ecology of goat.
3. To determine the effect of enzyme supplementation in TMR containing OPF silage on production cost of goat.

CHAPTER 2

Materials and Methods

2.1 Experimental site

The experiment was conducted at Thepa Research and Training Station, Klong Hoi Khong Research and Training Station, Faculty of Natural Resources, Prince of Songkla University and Laboratory of Feeds Quality Analysis, Department of Animal Science, Faculty of Natural Resources, Prince of Songkla University during November 2010 – November 2011.

2.2 OPF silage preparation

The fresh OPF gathered from Thepa Research and Training Station, Faculty of Natural Resources, Prince of Songkla University was chopped to 1 to 2 cm length and blend uniformly. Approximately 100 kg of the chopped OPF was packed in 150 l plastic drums without any preservation. The packed drums were tightly sealed to provide anaerobic conditions and kept at room temperature for 30 days before mixing with concentrate to form TMR.

2.3 Enzyme mixture

The enzyme product used in this study was obtained from National Center for Genetic Engineering and Biotechnology (BIOTEC), Thailand. It was a non-commercial product derived from *Aspergillus* spp. BCC 274, compliant with the current specifications for food-grade enzyme and generally recognized safe. The enzyme composition which measured at pH 6.8 were 1 X 10^7, 9 X 10^6, 2 X 10^6, 1 X

10^6 and 2 X 10^6 unit/kg dry weight for xylanase, β-glucanase, cellulase, mannanase, and amylase, respectively.

2.4 TMR preparation

The TMR used in the study was 60% (DM basis) OPF silage mixed with 40% (DM basis) concentrate (Table 5) which contained approximately 15% crude protein (CP) as recommended by NRC (1981) for goat and was typical of diets fed to goat. The ratio of OPF silage and concentrate of the TMR in the study related to Roddoung *et al*. (2010) who reported that TMR contained OPF silage and concentrate ratio of 50:50 showed the best result on average daily gain of Anglo Nubian X Thai Native male goat however, no significant difference was found regarding feed intake and feed conversion ratio when compared with the goat fed TMR contained OPF silage and concentrate ratio of 60:40. The concentrate was prepared as a loose mix then the enzyme was added to the concentrate portion before compositing with OPF silage. The TMR was prepared daily by hand.

Table 5. Ingredient composition of the TMR

Ingredient	g kg^{-1} of DM
OPF silage	600
Broken rice	148
Ground corn	125
Soybean meal	57
Fish meal	50
Urea	10
Dicalcium phosphate	5
Salt	5
Nutrient[1]	
CP (%)	15
Price of feed (baht/kg)[2]	3.98

[1]Calculated based on chemical composition of feedstuff from DLD (2004).
[2]Price of feed (baht/kg): oil palm frond silage 0.50, broken rice 11, ground corn 10, soybean meal 16, fish meal 30, urea 9.6, dicalcium phosphate 9, salt 5.

2.5 Animals and experimental design

Twenty four post-weaning Boer X TN crossbred male goats, 9 to 12 months of age with body weight (BW) of 11 to 18 kg were used in the experiment. All goats were drenched for internal worms (Invermectin, IDECTIN®, The British Dispensary, Co., Ltd.) prior to commencing the experiment. Each goat was placed in individual pen where water and mineral salt were available at all times. The animals were given 15 days of adaptation before the experiment. During the adaptation, the animals were offered a diet of fresh plicatulum grass *ad libitum* and concentrate (15% CP) at 1% of BW on DM basis.

The feeding trial was conducted using randomized complete block design (RCBD) of 4 treatments and 6 replications according to the body weight of goat. The treatments were according to enzyme supplementation levels as follows:

Treatment 1: TMR + enzyme 0 g/kg DM of TMR

Treatment 2: TMR + enzyme 2 g/kg DM of TMR

Treatment 3: TMR + enzyme 4 g/kg DM of TMR

Treatment 4: TMR + enzyme 6 g/kg DM of TMR

2.6 Experimental procedure, data collection, and sampling technique

The experiment was conducted for 90 days, with 15 days for adaptation period and 75 days for data and sample collection. During the adaptation period, the TMR was fed *ad libitum*, allowing for 10% refusal, twice daily in two equal portions at 08.30 and 14.00 h and voluntary feed intake (VFI) was determined. Fresh water was available at all times. In the data and sample collection period, the animals were randomly re-allocated to the four diets in the same manner as in the adaptation period. The amount of TMR offered was adjusted every 15 days according to the weight of each animal. The weights of TMR offered and that voided by individual goat during the 75 days collection period were recorded and representative samples were taken. The samples were oven dried at 65^{o}C for 72 hours and ground to pass through a 1 mm sieve for chemical analysis. Individual sample of TMR was collected three times each week and composited weekly for DM determination.

During the last six days of the data collection period, about 300 g of fecal samples from the rectum were collected from each animal twice daily in the morning and in the evening. The samples were bulked by animal, then oven dried at 65^{o}C for 48 hours and ground to pass through a 1 mm sieve for determination of apparent digestibility using acid insoluble ash (AIA) as an internal marker. On the last day of sample collection period, rumen fluid sample was collected at 0 h and 4 h post-

feeding, using a stomach tube connected with a vacuum pump. The pH of the rumen samples was measured immediately by a pH electrode MP. 125 LE 413 (Mettler Toleds, AG). Rumen fluid samples were then strained through two cheesecloths to remove particular matter. Samples were divided into two portions. One portion was used for ammonia nitrogen (NH_3-N) and VFAs analysis where 1 ml of H_2SO_4 (1 M) was added to 10 ml of rumen fluid. The mixture was centrifuged at 3,500 rpm for 15 minutes and supernatant was stored at -20^oC prior to NH_3-N and VFAs analysis. Another portion was fixed with 10% formaline solution in normal saline (0.9% NaCl) for total direct count of the bacteria, protozoa, and fungi zoospores where 1 ml formaline was added to 9 ml of rumen fluid. Blood samples were collected from the jugular vein at the same time of rumen fluid sampling. Blood samples were divided into two portions. The first portion was collected into an ethylenediaminetetraacetic acid (EDTA) coated tube for pack cell volume (PCV) determination. The second portion was collected into a plastic tube for blood urea nitrogen (BUN) analysis. The goat was weighed every two weeks before feeding in the morning during the data collection period for daily gain and feed conversion ratio calculation. Feed cost was also recorded for determination of cost production and feed cost per kilogram weight gain.

2.7 Analytical Procedures

Feed, refusal and feces were chemically analyzed using proximate analysis according AOAC (1990), NDF, ADF and ADL according to Goering and Van Soest (1970). AIA in feed and feces was analyzed and nutrient apparent digestibility coefficient was calculated using the method described by Van Keulen

and Young (1977). Ruminal VFAs analysis using a gas chromatography (GC 6890, Agilent Technologies) according to Josefa *et al*. (1999) and for NH_3-N using macro Kjeldahl method (AOAC, 1990). The total direct count of bacteria, protozoa, and fungi zoospores was made using the methods of Galyean (1989) based on the use of haemacytometer (Boeco) under a light microscope (Olympus CX31, Olympus Optical Co. Ltd.). BUN was measured using diagnostic kits (Enzymatetic Colorimetrict Test, Human Gesellschaftfur Biochemica und Diagnostica mbH) and PCV was measured by centrifuged (Haematocrit 24).

2.8 Statistical Analysis

All data was subjected to analysis of variance using general linear model (GLM) procedure of SAS (2008). Differences were tested using the PDIFF option and were declared significant at $P < 0.05$. Orthogonal polynomial contrasts were used to estimate the trend of the effect of enzyme supplementation level.

CHAPTER 3

Results

3.1. Chemical composition of OPF silage and TMR

The ingredients of TMR and calculated nutritive value of TMR are shown in Table 6 and chemical composition of OPF silage used in this study in Table 7. Then, the chemical composition of TMR supplemented with enzyme at 0, 2, 4, and 6 g/kgDM is shown in Table 8 .

Table 6. Nutritive value of ingredients of TMR (% DM basis)

Ingredient	DM	CP	ME[3]	Amount	CP	ME
OPF silage[1]	47.96	7.75	1.14	60.0	4.65	0.68
Broken rice[2]	87.60	7.80	3.24	14.8	1.15	0.48
Ground corn[2]	87.40	8.30	3.28	12.5	1.04	0.41
Soybean meal[2]	88.50	47.00	2.98	5.7	2.68	0.17
Fish meal[2]	89.80	60.00	1.43	5.0	3.00	0.07
Urea[2]	99.90	287.50	-	1.0	2.88	-
Dicalcium phosphate	-	-	-	0.5	-	-
Salt	-	-	-	0.5	-	-
Total				100.0	15.40	1.81

[1] Rattanagoson (2009)
[2] DLD (2004)
[3] Mcal/kgDM

Table 7. Chemical composition of OPF silage (% DM basis) used in this study

Composition	
DM (fresh)	41.22
DM (air dry)	93.67
OM	98.77
Ash	1.23
CP	4.12
EE	1.63
CF	41.01
NFE[1]	52.01
NDF	76.19
ADF	58.40
ADL	22.47

[1]NFE=100-(%CP+%EE+%CF +%Ash)

Table 8. Chemical composition of TMR supplemented with different levels of enzyme (% DM basis)

Items	Level of enzyme (g/kgDM of TMR)			
	0	2	4	6
DM	95.85	95.92	96.07	96.21
OM	92.07	91.47	90.39	92.85
Ash	7.93	8.53	9.61	7.15
CP	14.76	14.79	14.89	14.84
EE	1.72	2.14	1.93	1.92
CF	22.78	22.46	24.82	23.09
NFE	52.81	52.08	48.75	53.00
NDF	59.95	51.81	53.70	59.04
ADF	36.62	35.48	37.02	36.88
ADL	10.65	10.98	10.62	11.96

The TMR used in the present study contained OPF silage: concentrate ratio of 60:40 and a calculated CP content and ME were 15% and 1.81 Mcal/kgDM, respectively (Table 6). OPF silage used in this study contained CP that was lower (4.12%) than the expected value (7.75%). Meanwhile, pH of OPF silage was 4.2 and the silage was of good quality.

The TMR supplemented with different levels of enzyme had similar DM, OM, Ash, CP, EE, CF, ADF, and ADL content (Table 8). The CP content ranged from 14.76 to 14.89% (averaged 14.83%) which was slightly lower than the calculated CP level. The enzyme treatment at 2 and 4 g/kg DM, however, tended to result in a decreased NDF content of the TMR.

3.2. Effects of enzyme supplementation in TMR on intake and goat performance

The effects of enzyme supplementation in TMR on daily nutrient intake of goats are presented in Table 9. In this study, although the feed was offered *ad libitum* and the selection during feeding time was occurred, DMI, OM intake

(OMI), CP intake (CPI), and ADF intake (ADFI) were similar among treatments. However, NDF intake (NDFI) of goats receiving TMR supplemented with enzyme at 0 and 6 g/kgDM (P>0.05) was significantly higher (P<0.05) than those of goats receiving TMR supplemented with enzyme at 2 and 4 g/kgDM. The NDFI expressed either as total daily intake or based on metabolic BW, decreased quadratically (P<0.01) by increasing level of enzyme.

Table 9. Effect of enzyme supplementation in TMR on feed intake and nutrient intake of goat

Items	Level of enzyme (g/kgDM of TMR)				SEM[1]	Contrast[2]		
	0	2	4	6		L	Q	C
DMI								
g/h/d	442.89	437.26	422.46	434.57	10.99	ns	ns	ns
%BW	2.88	2.83	2.84	2.86	0.06	ns	ns	ns
g/kgBW$^{0.75}$/d	57.04	56.04	55.64	56.48	1.29	ns	ns	ns
OMI								
g/h/d	404.11	396.88	377.02	400.98	10.46	ns	ns	ns
g/kgBW$^{0.75}$/d	50.62	49.81	48.41	50.68	1.28	ns	ns	ns
CPI								
g/h/d	73.53	72.57	71.97	72.28	0.97	ns	ns	ns
g/kgBW$^{0.75}$/d	9.22	9.13	9.25	9.12	0.10	ns	ns	ns
NDFI								
g/h/d	246.78^{a}	201.85^{b}	196.21^{b}	235.65^{a}	9.18	ns	**	ns
g/kgBW$^{0.75}$/d	30.89^{a}	25.24^{b}	25.16^{b}	29.81^{a}	1.10	ns	**	ns
ADFI								
g/h/d	125.80	127.51	124.56	131.07	7.06	ns	ns	ns
g/kgBW$^{0.75}$/d	15.71	15.89	15.94	16.56	0.85	ns	ns	ns

[1]SEM: standard error of the means
[2]Contrast effects (L=linear, Q=quadratic, C=cubic)
[a-d] Means with different superscript within the same row are significantly different (P<0.05)
* P<0.05; ** P<0.01; ns: not significantly different (P>0.05)

Table 10 presents the effect of enzyme supplementation in TMR on growth performance of goat. There was no statistically significant difference (P>0.05) among treatments regarding weight gain and ADG, but goats receiving TMR supplemented with enzyme at 2 g/kgDM had numerically higher weight gain than the

goats receiving TMR supplemented with enzyme at 0, 4, 6 g/kgDM. Similarly, the ADG of goat receiving TMR supplemented with enzyme at 2 g/kgDM was numerically greater than those of goats receiving TMR supplemented with enzyme at 0, 4 and 6 g enzyme/kgDM. Moreover, the calculation of feed per gain revealed that goats receiving TMR supplemented with enzyme at 2 g/kgDM had the best feed per gain, followed by the goats receiving TMR supplemented with enzyme at 0, 4, and 6 g/kgDM.

Table 10. Effect of enzyme supplementation in TMR on growth performance of goat

Items	Level of enzyme (g/kgDM of TMR)				SEM[1]	Contrast[2]		
	0	2	4	6		L	Q	C
Body weight/BW (kg)								
Initial weight	13.90	13.60	13.66	14.00	0.14	ns	ns	ns
Final weight	16.86	17.28	16.29	16.63	0.25	ns	ns	ns
Weight gain	2.97	3.67	2.63	2.63	0.26	ns	ns	ns
ADG (g/d)	32.96	40.86	29.27	29.26	2.92	ns	ns	ns
Feed per gain	14.36	10.76	14.79	17.02	1.33	ns	ns	ns

[1]SEM: standard error of the means
[2]Contrast effects (L=linear, Q=quadratic, C=cubic)
ns: not significantly different (P>0.05)

3.3. Effect of enzyme supplementation in TMR on nutrient digestibility

The effects of supplementation of enzyme on apparent digestibility and digestible nutrient intake of goat are reported in Table 11. The DM, OM, and CP digestibility coefficient of TMR was not significantly affected by enzyme supplementation. Nevertheless, percentage of NDF and ADF digestibility coefficient was different among treatments (P<0.05). A quadratic effect of enzyme supplementation on NDF digestibility coefficient was observed. Goat receiving TMR supplemented with enzyme at 2 g/kgDM showed the lowest NDF digestibility

coefficient while no significant different was found regarding NDF digestibility coefficient between treatment with enzyme supplementation at 0, 4, and 6 g/kgDM. Increasing level of enzyme supplementation in TMR resulted in a linear and cubic ($P<0.05$) increase in digestibility coefficient of ADF. The ADF digestibility coefficient increased by enzyme supplementation up to 4 g/kgDM of TMR and slightly decreased by level of enzyme at 6 g/kgDM of TMR.

There were no significantly differences among treatments regarding intake of digestible DM and OM. The level of enzyme supplementation highly affected intake of digestible CP, NDF, and ADF. Goat receiving TMR supplemented with enzyme at 4 g/kgDM showed the highest intake of digestible CP and ADF ($P<0.05$). Meanwhile, a quadratic effect of enzyme supplementation on digestible NDF intake was observed. The digestible NDF intake of goat receiving TMR supplemented with enzyme at 0 and 6 g/kgDM ($P>0.05$) was significantly higher than that of goat receiving TMR supplemented with enzyme at 2 and 4 g/kgDM ($P<0.05$).

Enzyme supplementation significantly affected metabolizable energy intake (MEI) expressed based on the DMI (Mcal/kgDM). Goat receiving TMR supplemented with enzyme at 4 g/kgDM had the highest energy intake (1.83 Mcal/kgDM).

Table 11. Effect of enzyme supplementation in TMR on apparent digestibility and digestible nutrient intake of goat

Items	Level of enzyme (g/kgDM of TMR)				SEM	Contrast[2]		
	0	2	4	6		L	Q	C
Digestibility (%)								
DM	47.40	46.50	50.82	47.11	1.68	ns	ns	ns
OM	51.09	49.80	53.54	51.29	1.83	ns	ns	ns
CP	52.13	53.81	57.02	53.41	1.83	ns	ns	ns
NDF	35.73^{b}	22.38^{a}	30.86^{b}	33.77^{b}	2.42	ns	**	ns
ADF	11.09^{b}	11.62^{b}	22.22^{a}	16.71^{ab}	2.65	**	ns	**
Digestible nutrient intake								
DM								
g/d	212.25	205.90	216.87	207.00	5.29	ns	ns	ns
$g/kgBW^{0.75}/d$	26.59	25.84	27.88	26.16	0.65	ns	ns	**
OM								
g/d	208.73	200.02	203.97	207.88	5.37	ns	ns	ns
$g/kgBW^{0.75}/d$	26.14	25.09	26.21	26.27	0.67	ns	ns	ns
CP								
g/d	38.33^{b}	39.05^{b}	41.00^{a}	38.60^{b}	0.51	ns	ns	ns
$g/kgBW^{0.75}/d$	4.81^{b}	4.91^{b}	5.28^{a}	4.87^{b}	0.05	*	**	**
NDF								
g/d	88.17^{a}	45.48^{c}	60.58^{b}	79.58^{a}	2.99	ns	**	ns
$g/kgBW^{0.75}/d$	11.03^{a}	5.66^{c}	7.77^{b}	10.06^{a}	0.35	ns	**	**
ADF								
g/d	13.95^{c}	14.78^{c}	27.58^{a}	21.90^{b}	1.12	**	**	ns
$g/kgBW^{0.75}/d$	1.74^{c}	1.83^{c}	3.55^{a}	2.77^{b}	0.13	**	**	**
ME intake[3]								
Mcal/d	0.79	0.76	0.77	0.79	0.02	ns	ns	ns
Mcal/kgDM	1.79^{c}	1.74^{d}	1.83^{a}	1.81^{b}	0.003	**	**	**

[1]SEM: standard error of the means
[2]Contrast effects (L=linear, Q=quadratic, C=cubic)
[3]ME intake = 3.8XDOMI (Kearl, 1982)
[a-c] Means with different superscript within the same row are significantly different (P<0.05).
* P<0.05; ** P<0.01; ns: not significantly different (P>0.05)

3.4. Effect of enzyme supplementation in TMR on rumen fermentation process

Rumen fermentation parameters were measured for pH, NH_3-N and VFAs profile. In addition, BUN was determined to investigate their relationship with rumen NH_3-N concentration. The pattern of ruminal fermentation at 0 h and 4 h post-feeding and overall means are given in Table 12. Rumen fluid pH at 0 h and 4 h post-

feeding (7.75 to 7.88 and 7.35 to 7.44, respectively) and overall means (7.56 to 7.66) were unchanged by dietary treatments, while at 4 h after the onset of feeding, rumen pH declined as active fermentation of the newly ingested feed occurred.

Ruminal NH_3-N concentration at 4 h post-feeding was similar among treatments, except at 0 h post-feeding and overall means were affected ($P<0.05$) by treatments, ranging from 12.43 to 16.90 and 12.56 to15.86 mg/dl, respectively, and were significantly decreased by enzyme supplementation at 2 g/kgDM.

The effect of enzyme supplementation in TMR on production of total VFAs concentration, acetic acid (C_2), propionic acid (C_3) and butyric acid (C_4) proportion are shown in Table 13. Overall means of total VFAs in the rumen were not affected by dietary treatments. However, the concentration of total VFAs at 4 h post-feeding was significantly higher for goats receiving TMR without enzyme supplementation as compared with the goats receiving TMR with enzyme supplementation.

Table 12. Effect of enzyme supplementation in TMR on rumen fermentation characters of goat

Items	Level of enzyme (g/kgDM of TMR)				SEM[1]	Contrast[2]		
	0	2	4	6		L	Q	C
Ruminal pH								
0 h, post-feeding	7.75	7.84	7.88	7.81	0.05	ns	ns	ns
4 h	7.38	7.38	7.44	7.35	0.04	ns	ns	ns
Mean	7.56	7.60	7.66	7.58	0.10	ns	ns	ns
NH_3-N (mg/dl)								
0 h, post-feeding	16.67[a]	12.43[b]	15.79[b]	16.90[a]	0.81	ns	*	ns
4 h	15.05	12.69	13.92	13.58	0.82	ns	ns	ns
Mean	15.83[a]	12.56[b]	14.86[a]	15.24[a]	0.94	ns	ns	ns
BUN (mg/dl)								
0 h, post-feeding	28.78	27.05	27.33	28.06	1.62	ns	ns	ns
4 h	32.65	33.12	32.02	32.26	1.00	ns	ns	ns
Mean	30.72	30.08	29.67	30.16	1.60	ns	ns	ns

[1]SEM: standard error of the means
[2]Contrast effects (L=linear, Q=quadratic, C=cubic)
[a-b] Means with different superscript within the same row are significantly different (P<0.05).
* P<0.05; ns: not significantly different (P>0.05)

The molar proportion of C_2, C_3 and C_4 among treatments were similar (P>0.05), except the proportion of C_2 at 0 h post-feeding which was significantly higher for goats receiving TMR supplemented with enzyme at 2, 4 and 6 g/kgDM as compared with the goat receiving TMR without enzyme supplementation. The molar proportion of C_2 showed a linear increase while those of C_3 and C_4 showed a linear decrease in response to enzyme supplementation. Moreover, the C_2:C_3 ratio was similar (P>0.05) among dietary treatments and it was linear increase with enzyme supplementation.

Table 13. Effect of enzyme supplementation in TMR on ruminal VFA proportion of goat

Items	Level of enzyme (g/kgDM of TMR)				SEM[1]	Contrast[2]		
	0	2	4	6		L	Q	C
Total VFA (mmol/L)								
0 h, post-feeding	25.83	23.28	24.14	25.10	0.98	ns	ns	ns
4 h	41.20[a]	33.59[b]	35.35[b]	34.88[b]	0.87	**	**	*
Mean	33.52	28.44	29.74	29.99	2.86	ns	ns	ns
Acetate (mol/100mol)								
0 h, post-feeding	72.07[b]	73.98[a]	74.62[a]	74.57[a]	0.52	**	ns	ns
4 h	73.17	73.07	72.52	73.80	0.89	ns	ns	ns
Mean	72.62	73.52	73.56	74.18	0.70	*	ns	ns
Propionate (mol/100mol)								
0 h, post-feeding	14.84	14.67	13.71	14.30	0.35	ns	ns	ns
4 h	15.17	15.46	14.40	14.56	0.54	ns	ns	ns
Mean	15.01	15.06	14.06	14.43	0.42	*	ns	ns
Butyrate (mol/100mol)								
0 h, post-feeding	13.07	11.34	11.66	11.12	0.51	**	ns	ns
4 h	11.65	11.46	13.07	11.63	0.70	ns	ns	ns
Mean	12.36	11.40	12.36	11.38	0.59	ns	ns	*
C_2:C_3 ratio								
0 h, post-feeding	4.87	5.06	5.46	5.22	0.14	**	ns	ns
4 h	4.83	4.76	5.08	5.13	0.22	ns	ns	ns
Mean	4.85	4.90	5.27	5.18	0.17	*	ns	ns

[1]SEM: standard error of the means
[2]Contrast effects (L=linear, Q=quadratic, C=cubic)
[a-b] Means with different superscript within the same row are significantly different (P<0.05)
* P<0.05; ** P<0.01; ns: not significantly different (P>0.05)

3.5 Effect of enzyme supplementation in TMR on rumen microbial population

Table 14 illustrates the effect of enzyme supplementation in TMR on the population of rumen bacteria, protozoa, and fungi zoospores. Generally, the number of rumen microbes at 0 h post-feeding was greater than those of 4 h post-feeding. Craig *et al*. (1987) suggested that the decline might be due to a large proportion of ruminal bacteria becoming attached tenaciously to feed particles and not being dislodged by the typical procedure of blending before straining through cheesecloth. Dehority and Orpin (1997) explained that the peak number of rumen

bacteria occurred at feeding time and gradually diminished until 20 h after feeding. Population of rumen bacteria and fungal zoospores were not affected ($P>0.05$) by dietary treatment, although the bacteria population at 0 h post-feeding was significantly lower for goat receiving TMR supplemented with enzyme at 4 g/kgDM as compared with other treatments. Enzyme supplementation caused a linear increase in total protozoa count at 4 h post-feeding and overall mean. The same trend was also found with the population of both *Holotrich* sp and *Entodiniomorphs* sp. Protozoa population was enhanced in goat receiving TMR supplemented with enzyme at 4 and 6 g/kgDM as compared with other treatments.

Table 14. Effect of enzyme supplementation in TMR on rumen microbial population of goat

Items	Level of enzyme (g/kgDM of TMR)				SEM[1]	Contrast[2]		
	0	2	4	6		L	Q	C
Total direct count								
Bacteria (x10^{10} cell/ml)								
0 h, post-feeding	1.48^{a}	1.68^{a}	1.05^{b}	1.48^{a}	0.12	ns	ns	*
4 h	1.07	1.22	1.22	1.61	0.15	ns	ns	ns
Mean	1.27	1.45	1.14	1.55	0.16	ns	ns	*
Total Protozoa (x10^{6} cell/ml)								
0 h, post-feeding	1.66	1.71	3.48	1.88	0.59	ns	ns	ns
4 h	1.10^{c}	1.49^{b}	2.07^{ab}	2.73^{a}	0.25	**	ns	ns
Mean	1.38^{b}	1.60^{b}	2.77^{a}	2.30^{ab}	0.60	*	ns	ns
Holotrich sp. (x10^{5} cell/ml)								
0 h, post-feeding	1.41	1.20	2.31	1.16	0.49	ns	ns	ns
4 h	1.75^{b}	2.78^{b}	3.24^{ab}	6.25^{a}	1.00	**	ns	ns
Mean	1.58	1.99	2.78	3.70	0.95	*	ns	ns
Entodiniomorphs sp. (x10^{6} cell/ml)								
0 h, post-feeding	1.51	1.59	3.25	1.75	0.56	ns	ns	ns
4 h	0.92^{c}	1.21^{bc}	1.74^{ab}	2.10^{a}	0.17	**	ns	ns
Mean	1.22^{b}	1.40^{b}	2.49^{a}	1.92^{ab}	0.42	*	ns	ns
Fungal zoospores (x10^{6} cell/ml)								
0 h, post-feeding	3.75	4.06	3.69	3.97	0.36	ns	ns	ns
4 h	5.74	5.82	5.81	6.93	0.82	ns	ns	ns
Mean	4.74	4.94	4.75	5.45	1.01	ns	ns	ns

[1]SEM: standard error of the means
[2]Contrast effects (L=linear, Q=quadratic, C=cubic)
[a-c]Means with different superscript within the same row was significantly different P<0.05
* P<0.05; ** P<0.01; ns: not significantly different (P>0.05)

3.6 Effect of enzyme supplementation in TMR on blood packed cell volume (PCV)

The addition of enzyme to concentrate portion in TMR had no effect (P>0.05) on the concentration of PCV (Table 15). Overall, the PCV value at 4 h post-feeding was lower than that of 0 h post-feeding. The mean PCV value of all treatments ranged from 26.73 to 29.16%.

Table 15. Effect of enzyme supplementation in TMR on percentage of PCV of goat.

Items	Level of enzyme (g/kgDM of TMR)				SEM[1]	Contrast[2]		
	0	2	4	6		L	Q	C
PCV (%)								
0 h, post-feeding	30.33	29.54	26.75	28.83	1.20	ns	ns	ns
4 h	28.00	27.29	26.72	26.83	1.15	ns	ns	ns
Mean	29.16	28.41	26.73	27.83	1.20	ns	ns	ns

[1]SEM: standard error of the means
[2]Contrast effects (L=linear, Q=quadratic, C=cubic)
ns: not significantly different (P>0.05)

3.7 Effect of enzyme supplementation in TMR on production cost of goat

Table 16 presents the production cost of rearing goats with TMR containing OPF silage supplementation with different levels of enzyme. Details of the calculation are given in Appendix C.

Table 16. Production cost of rearing goats with TMR containing OPF silage supplemented with enzyme

Items	Level of enzyme (g/kgDM of TMR)			
	0	2	4	6
Production cost (baht/head)				
- Cost of live goat	1,390.00	1,364.00	1,324.00	1,400.00
- Cost of feed	160.40	159.26	156.31	165.36
- Cost of mineral block	27.50	27.50	27.50	27.50
- Cost of labor	191.25	191.25	191.25	191.25
- Cost of deworming treatment	3.20	3.14	3.05	3.22
Total Cost (baht/head)	1,772.35	1,745.15	1,702.11	1,787.33
Sale price of live goat(baht/head)	2,192.67	2,225.00	2,085.20	2,162.33
Revenue (Profit)[1]	420.32	480.45	383.09	375.00
Revenue (Profit)[2]	611.57	671.70	574.34	566.25
Rearing cost/kg gain[1]	128.74	109.53	135.04	147.27
Rearing cost/kg gain[2]	64.34	54.57	66.74	74.56

[1] the calculation included cost of labor
[2] the calculation excluded cost of labor

Rearing goats with TMR containing OPF silage supplemented with enzyme at 0, 2, 4 and 6 g/kgDM had a profit during 90 days rearing. The range of profit was 375.00 to 480.45 baht/head when cost of labor was included. Rearing goat with TMR supplemented with enzyme at 2 g/kgDM had the highest profit when compared with the other treatments. In addition, the rearing cost per kg weight gain of the treatment with 2 g enzyme/kgDM was the lowest.

On the other hand, if the cost of production excluded the labor cost, because usually the labor was the farmers and their family, rearing goats with TMR supplementation with different enzyme levels had profit ranging from 566.25 to 671.70 baht/head. In addition, the rearing cost per kg weight gain was the lowest when goat receiving TMR supplemented with enzyme at 2 g/kgDM (54.57 baht/kg gain).

CHAPTER 4

Discussion

4.1. Chemical composition of OPF silage and TMR

The CP content of OPF silage used in this experiment was much lower than the expectation. According to Dahlan *et al*. (2000), CP of OPF silage was 10.31%. Contrastly, Kawamoto *et al*. (2001) reported that OPF silage had 4.7% of CP. The significantly different CP content of forage depended on morphological status and plant age (Bilal *et al*., 2001). It was possible that OPF silage used in the present study contained more petiole than leaflet and also consisted of mature OPF. Consequently, the CP content of OPF silage decreased. On the other hand, the pH of OPF silage was 4.2 (acidic). The pH value indicated the quality of OPF silage. This result was in line with Kawamoto *et al*. (2001) that the low pH suggested that the silage was dominated by lactic acid bacteria which inhibited the losses of nutrient.

The proportion of forage and concentrate of TMR used in the present study was 60:40. This high forage diet was used in order to evaluate the effects of the enzyme on fiber digestion. Diets were formulated to be 15% CP (DM basis). Slightly lower concentration of CP in DM offered (14.83%) may have been because of lower percentage of CP level than expected in OPF silage and some ingredients or inconsistencies in TMR mixing or sampling. The CP value, almost 15%, is enough for fattening goat to achieved 50 g ADG (NRC, 1981).

The enzyme treatment, however, tended to decrease NDF content of the TMR, indicating that a partial hydrolysis of the fiber resulted from enzyme supplementation. In accordance with our study, Krause *et al*. (1998) reported that

fiber content of the TMR consisting of barley silage and barley based concentrate decreased after the enzyme (Pro-Mote, Biovance Technol. Inc., Omaha, NE) was applied to concentrate portion. It was unlikely that the fibrolytic enzymes hydrolyzed the fiber in the TMR during storage because they were stored in a dry state which should have precluded enzyme. The low NDF content of the TMR supplemented with enzyme might be due to the enzyme increased the susceptibility of the diet to the detergents used in fiber analysis (Krause *et al*., 1998). Contrastly, Hristov *et al*. (1998a) reported that the lowered NDF content in TMR consisting of rolled barley grain, corn silage, and soybean meal, treated with exogenous polysaccharide degrading enzyme (FinFeeds International Ltd, Malborough, U.K) compared to untreated TMR was as a result of enzymatic solubilization of plant fibers. Whether the decreasing NDF content caused by enzyme supplementation occurred before consumption or during the analytical procedure for fiber measurement is not known. Other researchers did not find any biologically effects on the chemical composition of the TMR (Beauchemin *et al*., 2000; Kung *et al*., 2000; 2002). The ADL content in TMR in this study was relatively high (10.62 to 11.96) which might have an effect on feed intake and digestibility (Hart and Wanapat, 1992; Van Soest, 1994). Chanjula *et al*. (2007) reported that the goat have limited rumen capacity to use highly lignified feed.

4.2. Effects of enzyme supplementation in TMR on intake and growth performance

DMI, OMI, CPI, and ADFI were similar among the treatments, except NDFI which was affected quadratically by enzyme supplementation. The goat in all

treatments consumed DM (%BW) less than the required DMI for goat in tropical region which was 3 to 3.1 %BW reported by Devendra and McLeroy (1982) and 3.05 to 3.66% of BW by Ashok and Wadhwani (1992). Furthermore AFRC (1998) recommended that the level of DMI for growing goats per $kgBW^{0.75}$ was 66 g $DM/kgBW^{0.75}$. It means that the DMI of the goats in this study was less than the recommended level for growing goat. Allison (1985) cited by Kawas *et al*. (1999) reported that the low nutrient intake was the most important factor limiting performance which explained the lower weight gain and ADG of goat than the target ADG (50 g/d) in this study. It is possible that low DMI could have been attributed to a high ADL (Hart and Wanapat, 1992; Wanapat, 2000) with low fermentation rate and digestibility leading to a low rate of disappearance through digestion passage and limited feed intake.

The study conducted by Yang *et al*. (2000) regarding the applied an enzyme product (Biovance Technologies Inc., Omaha, NE) with relatively high xylanase and low cellulase activities to the TMR (E-TMR) or to the barley-based concentrate portion (E-Conc) also showed that there were no significantly increase in the intake of DM, OM, NDF, ADF, and CP in dairy cows. Others, using different enzyme products, have reported no differences in DMI of dairy cows (Schinogethe *et al*., 1999), feedlot cattle (McAllister *et al*., 1999), goats (Titi and Lubbadeh, 2004) or sheep (Giraldo *et al*., 2008) and increased feed intake of dairy cows (Beauchemin *et al*., 2000) and lambs (Pinos-Rodriguez *et al*., 2002). The effects of enzyme products on DMI appear to differ among enzyme products but the method of applying enzymes to diet is apparently not a major factor influence feed intake (Yang *et al*., 2000). Feng *et al*. (1996) reported that DMI was increased by fibrolytic enzyme with dry, but not

fresh forages. However, some studies reported that fibrolytic enzymes sprinkled on forages (Lewis *et al*., 1996; Krause *et al*., 1998), directly fed to the animal (Lewis *et al*., 1996), or added to the feed (Rode *et al*., 1999) did not change DMI. In the present study, although DMI did not increase, the addition of enzyme to the TMR tended to improve the ADG and weight gain ($P<0.07$). Low level of enzyme supplementation (2 g/kgDM) gave the best ADG (40.86 g/d) and weight gain (3.67 kg). The ADG tended to decrease with increasing enzyme levels.

4.3. Effect of enzyme supplementation in TMR on nutrient digestibility

In the present experiment, apparent digestibility of DM, OM, and CP were not affected by the supplementation of enzyme. This evidence was also found by McAllister *et al*. (1999) that the exogenous enzyme mixed to silage or delivered directly to the rumen did not affect intake and DM digestibility. Furthermore, exogenous enzyme has typically been observed to increase the initial rate but not to extent of DM digestion when used in ruminant diets (Feng *et al*., 1996; Wang and McAllister, 2002). Gilardo *et al*. (2008) declared that applying enzyme 12 g/d to the sheep did not affect either DMI or feed digestibility. In contrast, Beauchemin *et al*. (1995) claimed that the addition of fibrolytic enzyme increased feed digestibility of steers fed dry forages.

In the present study, supplementation of enzyme in TMR affected NDF and ADF digestibility. NDF digestibility quadratically increased and ADF digestibility showed a linear and cubic increase with the level of enzyme supplementation. Goat receiving TMR supplemented with enzyme at 2g/kgDM showed the lowest NDF digestibility and goat receiving TMR supplementation with

enzyme at 4 g/kgDM showed the highest ADF digestibility. These results are similar to the findings of Pinos-Rodriguez *et al*. (2002) who reported that the addition of enzyme improved NDF digestion in lambs fed alfalfa hay. Morgavi *et al*. (2000) suggested that interactions between the enzymes and substrate caused a "scarring" of the fiber particles that resulted in improved attachment by types of substrate.

The digestible DMI and OMI were not significantly affected by the addition of enzyme which were similar with the result of McAllister *et al*. (1999) that the digestible OMI did not differ from untreated treatment (no enzyme) and the value tended to decrease. The increase of digestible CPI was also observed in the present study which might be due to the supplementary effect of exogenous enzyme activity on the protease enzyme activity (McAllister *et al*., 1999). Although enzyme supplementation did not improve NDFI, the high digestible ADFI was observed for the high level of enzyme supplementation because of the combined effect of increase intake and digestion of ADF.

The MEI of goat receiving TMR supplemented with different levels of enzyme ranged from 0.76 to 0.79 Mcal/d or 1.74 to 1.83 Mcal/kgDM. According to NRC (1981), the MEI for growing goat which expected 50 g/d for ADG, is approximately 2.51 Mcal/kgDM. Hence, the MEI from TMR in this study did not fulfill the growing goat requirement. According to Roeder *et al*. (1997), the ADG of crossbred Boer (Boer X Spanish) was higher than indigenous Spanish goat, but under low quality and low availability of forage, the ADG was similar. In addition, the crossbred of Boer need longer period to adapt with low quality diets than the native goat (Joemat *et al*., 2004). Luo *et al*. (2003) reported that an energy requirement for gain (ME per unit of ADG) was 14% greater for growing goats with 50% or more

Boer breed compared with indigenous or local genotypes. In this study, goat receiving TMR supplemented with enzyme at 2 g/kgDM had the lowest MEI, however, they showed the best ADG and weight gain. Therefore, the addition of enzyme at low level to the diet improved goat performance.

The improvement of animal performance depended on the physiological status of the animal and the condition during the experiment. Although the increase of digestibility was occurred, the animal production did not improve suddenly. Beauchemin *et al*. (2003) stated that the improvements in animal performance due to the use of enzyme supplementation were attributed mainly to improvements in ruminal fiber digestion resulting in increased digestible energy intake. Ballard *et al*. (2003) also reported that the dry enzyme addition to lactating dairy cows ration improved the DM, OM, and non-fiber carbohydrate (NFC) degradation, but not affected milk yield and milk composition. Nsereko *et al*. (2002) declared that exogenous enzyme stimulated the increase of microbial population which consequently enhanced digestibility and animal performance. On the other hand, the increasing level of enzyme followed by the reducing animal performance. They also considered that the enzyme supplementation at low level to ruminant feed caused beneficial disruption of the surface structure of feed both before and after ingestion. However, when applying enzyme at an excess level, the beneficial breakdown of the feed surface may have been minimized due to the enzyme attached to feed may have restricted microbial attachment and limited digestion of feed. In general, animal responses were greatest when fiber digestion was compromised and when energy was the first-limiting nutrient in the ration.

4.4. Effect of enzyme supplementation in TMR on rumen fermentation process

Rumen fluid pH was unchanged by dietary treatment, indicating no specific effect of enzyme supplementation. In term of pH, Morgavi *et al*. (2000) observed a synergistic effect in the rumen between exogenous and ruminal bacterial enzyme with the greatest synergistic effect at pH range of 5.0 to 6.0. Level of pH is the critical factor for enzyme activity, lower or greater value of pH has the same effect in reducing the capability of enzyme to digest certain substrate (Campbell and Reece, 2008). Calsamiglia *et al*. (2002) reported that NDF digestibility decreased when fermenter pH was reduced from 6.4 to 5.7 in continuous culture. The rumen fluid pH in the present study (7.56 to 7.66) was, however, high when compared with the optimal pH (6.0-7.0) for microbial digestion of protein and fiber (Ørskov and Ryle, 1990; Van Soest, 1994). The relatively high rumen fluid pH observed might be caused by the contamination of saliva during rumen fluid sample collection.

Mean ruminal NH_3-N concentration was lower in goat receiving TMR with enzyme supplementation than those at of goats receiving TMR without enzyme supplementation. Ruminal NH_3-N concentration is considerably higher when measured before feeding compared to after feeding. Higher NH_3-N concentration before feeding reflects primarily a lack of synchrony between fermentable energy and protein (Beauchemin *et al*., 2000). In the present study, the low level of enzyme supplementation decreased NH_3-N concentration which was likely caused by an increase in ruminal availability of slowly digestible carbohydrate due to enzyme supplementation. Adesogan *et al*. (2007) also reported that an enhanced uptake of NH_3-N by the ruminal microbes was perhaps because of the availability of fermentable metabolizable energy from the diet. Concentration of ruminal NH_3-N in

the present study was higher than 5 to 8 mg/dl, which is the optimal level of NH_3-N for microbial protein synthesis (Satter and Slyter, 1974).

BUN value in this experiment was not different among the treatments. The entire goat obtained similar CP content, around 15%, in their diets. Hammond (1998) stated that in ruminant, BUN concentration was closely related to NH_3-N in rumen. Obara and Shimbayashi (1980) cited by Sun and Christopherson (2005) declared that the increased N intake in which BUN increased reached a stable level at approximately 30 mg/dl. However, BUN concentration may increase above that level if the sheep received low quality roughage diet. There are a complicated regulation of BUN involving influences of dietary N content including the type of diet, the availability of fermentable OM, ruminal NH_3-N concentration, and urea recycling. Observed BUN concentration in the present study was close to the optimal level which has been reported in the range of 11.2 to 27.7 mg/dl (Lloyd, 1982).

The amount of total VFAs reflected the fermentation activity in the rumen, the higher total VFAs means the more rumen fermentation activity (Abdullah *et al.*, 1995). The previous *in vivo* studies of exogenous enzyme utilization from many researchers (Hristov *et al*., 2000; Pinos-Rodriguez *et al*., 2002; Beauchemin *et al*., 2003) reported that treating different feed with fibrolytic enzymes changed rumen fermentation pattern. In the present study, the proportion of C_2, C_3, and C_4 among treatments were similar ($P>0.05$), except the proportion of C_2 at 0 h post-feeding which was significantly increased by enzyme supplementation ($P<0.05$). This finding was similar to that reported by Beauchemin *et al*. (2000) who showed that the proportion of C_2 was higher for cows fed the low level of enzyme (Natugrain 33-L; BASF corporation Ludwigshafen, Germany) compared with the control (no enzyme

supplementation). Boonthep *et al*. (2011) reported that there was significant difference in fermentable soluble fraction between untreated and enzyme treated TMR containing OPF silage. They informed that the increase of fermentable soluble fraction and potential of extent of gas production with enzyme treated TMR indicating an increase in the rate of fermentation and probably degradation of feedstuffs in the rumen compared with untreated TMR. These results support the observations of the present study that ruminal NH_3-N concentration was decreased with a low level of enzyme supplementation due to increasing ruminal availability of slowly digestible carbohydrate.

The C_2 to C_3 ratio tended to be slightly higher by inclusion of enzyme, the enzyme supplementation increased the daily output of C_2 without decreasing the production of C_3. The ratio of C_2, C_3, and C_4 in the present study was, however, in accordance with the reports by Bowen (2010) that the molar ratio of C_2 to C_3 to C_4 on diet with high crude fiber was roughly 70:20:10. According to Paengkoum *et al*. (2006), goat receiving 10-30 g urea/kg steamed OPF had the average of ratio of C_2: C_3: C_4 68:25:7. In addition, Giraldo *et al*. (2008) noticed that the supplementation of enzyme to the sheep fed 70:30 grass hay:concentrate diet had 68:20:12 of molar proportion of C_2, C_3, and C_4 at 4 h post feeding.

4.5 Effect of enzyme supplementation in TMR on rumen microbial population

Many researchers have shown that exogenous enzyme can enhance fiber degradation by ruminant microorganism (Hristov *et al*., 1998b; Feng *et al*., 1996), *in situ* (Feng *et al*., 1996; Lewis *et al*., 1996) and *in vivo* (Yang *et al*., 1999). Wang *et al*. (2001) also reported that enzyme supplementation increased numbers of non-fibrolytic and fibrolytic bacteria in a batch culture system using rumen fluid. Stimulation of rumen microbial numbers through the use of enzyme could result in greater microbial biomass, which would provide more total polysaccharidase activity to digest feedstuffs. Silva *et al*. (1987) cited by Chen *et al*. (2008) reported that the rate of fiber degradation depended on the extent to which the rumen environment allowed an adherent cellulolytic microbial population to develop. In the present study, there were no effects of enzyme supplementation on total bacteria population and fungi zoospore. However, protozoa population was enhanced in goat receiving TMR supplemented with enzyme at 4 and 6 g/kgDM. Enzyme supplementation caused a linear increase in protozoa population. Growth of protozoa depends on the availability of energy from sugar (holotrichs), starch, and probably cellulose and hemicellulose for entodiniomorphs that are able to attack those kinds of substrates (Van Soest, 1994). Jouany and Ushida (1999) also reported a strong correlation existed between the number of holotrichs and the content of sugars in the diet. Rojo *et al*. (2005) explained that protozoa can be stimulated by increasing rapidly fermentable starch in the diet until some point when acidic conditions affected them negatively. The treatment of concentrate portion of TMR with enzyme in the present study probably increased digestion of feedstuff, releasing more starch, and sugar into the rumen. This hypothesis is consistent with the finding of Yang *et al*. (1999) who reported a

numerically higher ruminal starch digestion but lower fiber digestion, when the enzyme mixture contained mainly xylanase, and cellulase activity, was incorporated into the entire diet than when the enzyme mixture was added to the forage portion of the diet. Releasing reducing sugars during the pretreatment of feeds with enzymes has been also proposed as a possible mode of action of fibrolytic enzymes (Nsereko *et al*., 2000; Beauchemin *et al*., 2003). In addition, the enzyme mixture used in the present study contained not only fibrolytic activity but also amylase activity, which enhanced starch degradation. Although sugar released after enzymes treatment was not measured in the present study, our results suggested that the increase of protozoa population might be caused by the availability of starch and sugar in the rumen.

Furthermore, the present study found that the total production of VFAs was in accordance with the increase of protozoa population. It was probably because of the diminishment of rumen microbial population, then influenced the fermentation rate in the rumen and reduced VFAs production. VFAs, carbon dioxide (CO_2), and methane (CH_4) are end products of fermentation process, particularly by rumen bacteria (Singh *et al*., 1977; Van Soest, 1994). When the presence of protozoa in the rumen was increased, the population of bacteria was decreased. It appears reasonable, because protozoa became predator for bacteria (Bird *et al*., 1979; Ffoulkes and Leng, 1988; Nolan *et al*., 1989).

4.6 Effect of enzyme supplementation in TMR on blood packed cell volume (PCV)

There was no significant difference among treatments regarding PCV level both at 0 h and 4 h post-feeding. The percentage of PCV at 4 h was slightly

lower than 0 h post-feeding measurement. But, overall PCV values were still in the normal range of 22 to 38% (Jain, 1993) which reflected the good health condition of goat throughout the study.

4.7 Effect of enzyme supplementation in TMR on production cost of goat

The estimation of production cost of goat in the present study considered direct cost such as the price of live goat, feed, mineral block, deworming, and labor. Chamdi (2004) stated that if all production cost including land, housing equipment, and capital was included in the calculation of the production cost, the small scale farmer only got a little profit or no profit at all.

In the present experiment, increasing of feed cost was concomitant with the increasing level of enzyme. It will become a problem for applying this innovation to the farmer because mostly the farmer considers the cost for adopting the new innovation. On the other hand, rearing goat with TMR supplemented with enzyme at 2 g/kg showed the highest profit and the cheapest cost per live weight gain. Furthermore, the rearing cost per kg weight gain reduced from 128.74 to 109.53 baht/head when rearing goat with TMR supplementation with enzyme at 2 g/kg relative to that of TMR with no enzyme supplementation (enzyme at 0 g/kg). It means that applying 2 g enzyme/kg gave more profit than other treatments.

Chamdi (2004) reported that the factors affecting the income was farming scale, land, capital, managing skills, the value of the livestock's products, livestock productivity, input cost, and the price of product. In the present study, six goats were reared in a treatment which was slightly higher than the average number of goats owned by small farmer in tropical region such as Thailand. Chaiyawan (2008)

reported that the number of goat owned by a small farmer was 5.3 head/family. According to Soedjana (1998) cited by Chamdi (2004) noticed that the number of goat owned by the farmer had positive effect on increasing the farmer's income. The production cost is more efficiently reduced as a number of goats increased.

CHAPTER 5

Conclusion and Recommendation

5.1 Conclusion

This study was designed to see the effects of rearing crossbred Boer X Thai Native male goat with TMR containing OPF silage supplemented with different levels of enzyme derived from *Aspergillus* spp. BCC 274, on feed intake, apparent digestibility, rumen ecology, growth performance, and cost production during 90 days. Supplementation of enzyme had no effects on DMI, OMI, CPI and ADFI, except NDFI which was affected by enzyme supplementation. Although DMI did not increase, goat receiving TMR supplemented with enzyme at 2 g/kgDM had numerically highest ADG and weight gain (40.86 g/d and 3.67 kg, respectively) and the best feed per gain (10.76).

The apparent digestibility coefficients of DM, OM, and CP did not affect by the supplementation of enzyme in TMR. A quadratic effect of enzyme supplementation on NDF digestibility coefficient was observed. Increasing level of enzyme supplementation resulted in a linear and cubic increase in ADF digestibility coefficient. Ruminal NH_3-N concentration was lower in goat receiving TMR supplemented with enzyme at 2 g/kgDM, indicating an increase in ruminal availability of slowly digestible carbohydrate. Furthermore, the proportion of C_2 linearly increased as level of enzyme supplementation increased. There were no effects of enzyme supplementation on bacteria and fungi zoospores. Enzyme supplementation, however, caused a linear increased in protozoa population . The treatment of TMR with enzyme probably increased digestion of feedstuff, releasing more starch and sugar into the rumen. Regarding the cost production, rearing goats

with TMR supplemented with enzyme at 2 g/kgDM had lower rearing cost per kg weight gain.

5.2 Recommendation

Within the present experiment the most favorable production responses, though non-significant, were obtained when the enzyme product was applied to concentrate portion of TMR at 2 g/kgDM. The experiment also provided clear evident that enzyme added to the TMR are capable of manipulating the rumen fermentation process in goat. However, further study is warranted to investigate effect of enzyme level lower than 2 g/kgDM on productive performance of goat, so that on farm efficacy of enzyme supplement can be assured. In addition, further study using a large number of goats fed for a longer duration with a good quality TMR are needed to confirm the effect of addition of enzyme.

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APPENDICES

Appendix A. Figures during the experiment period

OPF silage preparation

Figure 1. Fresh OPF

Figure 2. Chopped OPF

Figure 3. OPF silage

Figure 4. pH measurement of OPF silage

Figure 5. The surface of OPF silage (removed)

Figure 6. OPF silage (used)

TMR preparation

Figure 1. Enzyme OPF silage

Figure 2. Concentrate mixed with enzyme

Figure 3. TMR

Figure 4. Weighing for TMR

Figure 5. TMR for goat

The situation and activities at goat farm during experimental period

Figure 1. The barn for rearing goat

Figure 2. Individual pen

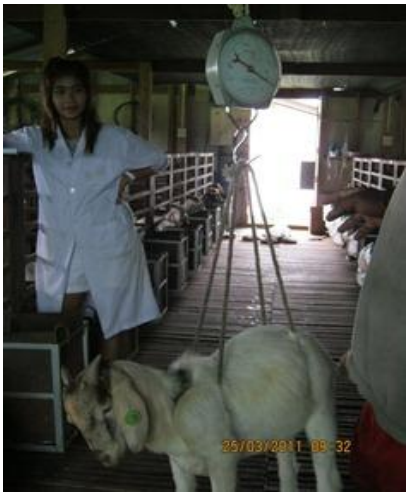

Figure 3.Weighing goat

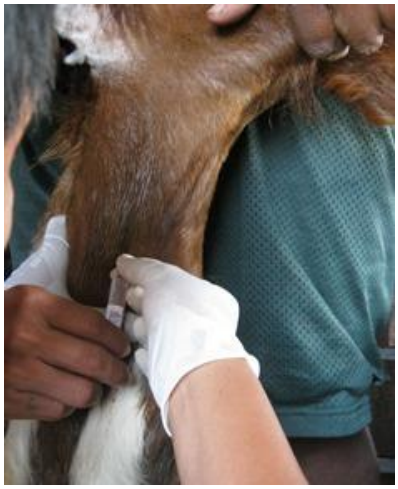

Figure 4. Blood sample collection

Figure 5. Blood sample

Figure 6. Rumen fluid sample collection

Figure 7. Rumen pH measurement

Figure 8. Rumen fluid sample

Figure 9. Feces sample collection for AIA analysis

Equipments and activities in Feed Analytical Laboratory

Figure 1. Oven 100ºC

Figure 2. Furnace 500-600 ºC

Figure 3. CP analysis

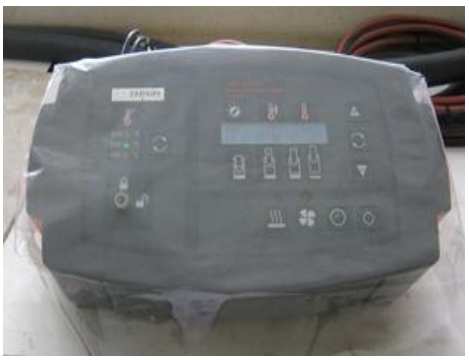

Figure 4. EE analysis

Figure 5. Equipment for CF, ADF, NDF and AIA analysis

Figure 6. Centrifuge

Figure 7. Microscope

Figure 8. Counting ruminal microbes equipments

Appendix B. Statistical Analysis (ANOVA)

Table B.1 Analysis of variance for the mean of DMI (g/h/d)

Source	DF	Sum of squares	Mean Square	P-value
Model	8	51484.31286	6435.53911	0.0004
Treatment	3	1138.18471	379.39490	0.6739
Block	5	49685.04721	9937.00944	<.0001
Error	13	9426.88402	725.14492	
Corrected Total	21	60911.19688		

Table B.2 Analysis of variance for the mean of DMI (%BW)

Source	DF	Sum of squares	Mean Square	P-value
Model	8	0.31155227	0.03894403	0.2472
Treatment	3	0.01164167	0.00388056	0.9281
Block	5	0.29531167	0.05906233	0.1077
Error	13	0.33717500	0.02593654	
Corrected Total	21	0.64872727		

Table B.3 Analysis of variance for the mean of DMI ($g/kgBW^{0.75}/d$)

Source	DF	Sum of squares	Mean Square	P-value
Model	8	29.1418269	3.6427284	0.9212
Treatment	3	5.83931786	1.94643929	0.8979
Block	5	22.63146786	4.52629357	0.8036
Error	13	129.7408321	9.9800640	
Corrected Total	21	158.8826591		

Table B.4 Analysis of variance for the mean of OMI (g/h/d)

Source	DF	Sum of squares	Mean Square	P-value
Model	8	44584.18746	5573.02343	0.0004
Treatment	3	2246.28451	748.76150	0.3693
Block	5	41349.30097	8269.86019	0.0001
Error	13	8535.36509	656.56655	
Corrected Total	21	53119.55255		

Table B.5 Analysis of variance for the mean of OMI (g/kgBW$^{0.75}$/d)

Source	DF	Sum of squares	Mean Square	P-value
Model	8	21.9855424	2.7481928	0.9621
Treatment	3	17.21895528	5.73965176	0.6385
Block	5	6.15883028	1.23176606	0.9843
Error	13	128.6660031	9.8973849	
Corrected Total	21	150.6515455		

Table B.6 Analysis of variance for the mean of CPI (g/h/d)

Source	DF	Sum of squares	Mean Square	P-value
Model	8	1227.908390	153.488549	<.0001
Treatment	3	7.679862	2.559954	0.7225
Block	5	1210.769608	242.153922	<.0001
Error	13	74.175155	5.705781	
Corrected Total	21	1302.083545		

Table B.7 Analysis of variance for the mean of CPI (g/kgBW$^{0.75}$/d)

Source	DF	Sum of squares	Mean Square	P-value
Model	8	0.45854186	0.05731773	0.5414
Treatment	3	0.06638504	0.02212835	0.7907
Block	5	0.34079004	0.06815801	0.4184
Error	13	0.82463996	0.06343384	
Corrected Total	21	1.28318182		

Table B.8 Analysis of variance for the mean NDFI (g/h/d)

Source	DF	Sum of squares	Mean Square	P-value
Model	8	25712.46622	3214.05828	0.0018
Treatment	3	9922.68269	3307.56090	0.0062
Block	5	14487.08150	2897.41630	0.0052
Error	13	6570.14642	505.39588	
Corrected Total	21	32282.61264		

Table B.9 Analysis of variance for the mean of NDFI (g/kgBW$^{0.75}$/d)

Source	DF	Sum of squares	Mean Square	P-value
Model	8	150.5309265	18.8163658	0.0637
Treatment	3	145.1397235	48.3799078	0.0060
Block	5	3.3893402	0.6778680	0.9920
Error	13	95.4704598	7.3438815	
Corrected Total	21	246.0013864		

Table B.10 Analysis of variance for the mean of ADFI (g/h/d)

Source	DF	Sum of squares	Mean Square	P-value
Model	8	7087.92240	885.99030	0.0398
Treatment	3	134.436974	44.812325	0.9279
Block	5	6888.347397	1377.669479	0.0122
Error	13	3884.88591	298.83738	
Corrected Total	21	10972.80831		

Table B.11 Analysis of variance for the mean of ADFI (g/kgBW$^{0.75}$/d)

Source	DF	Sum of squares	Mean Square	P-value
Model	8	7.47462141	0.93432768	0.9816
Treatment	3	2.40052671	0.80017557	0.9041
Block	5	5.11681504	1.02336301	0.9387
Error	13	55.95861496	4.30450884	
Corrected Total	21	63.43323636		

Table B.12 Analysis of variance for the mean of initial weight

Source	DF	Sum of squares	Mean Square	P-value
Model	8	118.8888880	14.8611110	<.0001
Treatment	3	0.5794940	0.1931647	0.2176
Block	5	117.0401607	23.4080321	<.0001
Error	13	1.4838393	0.1141415	
Corrected Total	21	120.3727273		

Table B.13 Analysis of variance for the mean of final weight

Source	DF	Sum of squares	Mean Square	P-value
Model	8	110.4416126	13.8052016	<.0001
Treatment	3	2.4379762	0.8126587	0.1369
Block	5	107.2046429	21.4409286	<.0001
Error	13	4.8020238	0.3693864	
Corrected Total	21	115.2436364		

Table B.14 Analysis of variance for the mean of weight gain

Source	DF	Sum of squares	Mean Square	P-value
Model	8	7.29903950	0.91237994	0.1007
Treatment	3	3.54782738	1.18260913	0.0791
Block	5	5.17916071	1.03583214	0.0863
Error	13	5.41550595	0.41657738	
Corrected Total	21	12.71454545		

Table B.15 Analysis of variance for the mean of ADG (g/d)

Source	DF	Sum of squares	Mean Square	P-value
Model	8	900.916383	112.614548	0.1008
Treatment	3	437.8766975	145.9588992	0.0792
Block	5	639.2909392	127.8581878	0.0864
Error	13	668.857544	51.450580	
Corrected Total	21	1569.773927		

Table B.16 Analysis of variance for the mean of feed per gain

Source	DF	Sum of squares	Mean Square	P-value
Model	8	296.5624475	37.0703059	0.0231
Treatment	3	104.5953354	34.8651118	0.0564
Block	5	212.8324188	42.5664838	0.0208
Error	13	139.1514479	10.7039575	
Corrected Total	21	435.7138955		

Table B.17 Analysis of variance for the mean of coefficient digestibility of DM

Source	DF	Sum of squares	Mean Square	P-value
Model	8	95.7841633	11.9730204	0.6844
Treatment	3	54.26718452	18.08906151	0.3984
Block	5	40.75533786	8.15106757	0.7858
Error	13	221.1931821	17.0148602	
Corrected Total	21	316.9773455		

Table B.18 Analysis of variance for the mean of coefficient digestibility of OM

Source	DF	Sum of squares	Mean Square	P-value
Model	8	74.0229305	9.2528663	0.8627
Treatment	3	33.97443810	11.32481270	0.6485
Block	5	36.26202143	7.25240429	0.8661
Error	13	261.1737786	20.0902907	
Corrected Total	21	335.1967091		

Table B.19 Analysis of variance for the mean of coefficient digestibility of CP

Source	DF	Sum of squares	Mean Square	P-value
Model	8	125.4210329	15.6776291	0.6278
Treatment	3	66.31380945	22.10460315	0.3842
Block	5	60.60526778	12.12105356	0.6989
Error	13	261.2278989	20.0944538	
Corrected Total	21	386.6489318		

Table B.20 Analysis of variance for the mean of coefficient digestibility of NDF

Source	DF	Sum of squares	Mean Square	P-value
Model	8	657.339960	82.167495	0.0844
Treatment	3	532.0078902	177.3359634	0.0157
Block	5	61.7307568	12.3461514	0.8731
Error	13	458.195276	35.245790	
Corrected Total	21	1115.535236		

Table B.21 Analysis of variance for the mean of coefficient digestibility of ADF

Source	DF	Sum of squares	Mean Square	P-value
Model	8	504.233227	63.029153	0.2513
Treatment	3	405.0730949	135.0243650	0.0595
Block	5	90.3313183	18.0662637	0.8220
Error	13	550.246355	42.326643	
Corrected Total	21	1054.479582		

Table B.22 Analysis of variance for the mean of digestible nutrient intake of DM (g/d)

Source	DF	Sum of squares	Mean Square	P-value
Model	8	12132.09695	1516.51212	0.0003
Treatment	3	379.70761	126.56920	0.5398
Block	5	11747.20882	2349.44176	<.0001
Error	13	2184.38000	168.02923	
Corrected Total	21	14316.47695		

Table B.23 Analysis of variance for the mean of digestible nutrient intake of DM ($g/kgBW^{0.75}/d$)

Source	DF	Sum of squares	Mean Square	P-value
Model	8	15.95758331	1.99469791	0.6235
Treatment	3	11.60415528	3.86805176	0.2550
Block	5	1.71645028	0.34329006	0.9812
Error	13	32.98860305	2.53758485	
Corrected Total	21	48.94618636		

Table B.24 Analysis of variance for the mean of digestible nutrient intake of OM (g/d)

Source	DF	Sum of squares	Mean Square	P-value
Model	8	11607.01729	1450.87716	0.0005
Treatment	3	251.10713	83.70238	0.6997
Block	5	11164.80184	2232.96037	0.0001
Error	13	2252.40686	173.26207	
Corrected Total	21	13859.42415		

Table B.25 Analysis of variance for the mean pf digestible nutrient intake of OM ($g/kgBW^{0.75}/d$)

Source	DF	Sum of squares	Mean Square	P-value
Model	8	7.39346946	0.92418368	0.9329
Treatment	3	4.60733385	1.53577795	0.6448
Block	5	1.57600885	0.31520177	0.9863
Error	13	35.04465781	2.69574291	
Corrected Total	21	42.43812727		

Table B.26 Analysis of variance for the mean of digestible nutrient intake of CP (g/d)

Source	DF	Sum of squares	Mean Square	P-value
Model	8	369.6869714	46.2108714	<.0001
Treatment	3	22.1644600	7.3881533	0.0213
Block	5	352.9833350	70.5966670	<.0001
Error	13	20.9684150	1.6129550	
Corrected Total	21	390.6553864		

Table B.27 Analysis of variance for the mean of digestible nutrient intake of CP ($g/kgBW^{0.75}/d$)

Source	DF	Sum of squares	Mean Square	P-value
Model	8	0.84574446	0.10571806	0.0029
Treatment	3	0.66102552	0.22034184	0.0005
Block	5	0.09168719	0.01833744	0.4593
Error	13	0.24018281	0.01847560	
Corrected Total	21	1.08592727		

Table B.28 Analysis of variance for the mean of digestible nutrient intake of NDF (g/d)

Source	DF	Sum of squares	Mean Square	P-value
Model	8	7594.629032	949.328629	<.0001
Treatment	3	5829.484038	1943.161346	<.0001
Block	5	1341.862438	268.372488	0.0091
Error	13	700.482695	53.883284	
Corrected Total	21	8295.111727		

Table B.29 Analysis of variance for the mean of digestible nutrient intake of NDF ($g/kgBW^{0.75}/d$)

Source	DF	Sum of squares	Mean Square	P-value
Model	8	95.2492661	11.9061583	<.0001
Treatment	3	91.64711310	30.54903770	<.0001
Block	5	0.40864310	0.08172862	0.9882
Error	13	9.6871202	0.7451631	
Corrected Total	21	104.9363864		

Table B.30 Analysis of variance for the mean of digestible nutrient intake of ADF (g/d)

Source	DF	Sum of squares	Mean Square	P-value
Model	8	783.5896931	97.9487116	<.0001
Treatment	3	628.5602196	209.5200732	<.0001
Block	5	150.4002179	30.0800436	0.0208
Error	13	98.4130388	7.5702338	
Corrected Total	21	882.0027318		

Table B.31 Analysis of variance for the mean of digestible nutrient intake of ADF ($g/kgBW^{0.75}/d$)

Source	DF	Sum of squares	Mean Square	P-value
Model	8	11.48794354	1.43599294	<.0001
Treatment	3	11.06164278	3.68721426	<.0001
Block	5	0.09820112	0.01964022	0.9645
Error	13	1.40356555	0.10796658	
Corrected Total	21	12.89150909		

Table B.32 Analysis of variance for the mean of ME intake (Mcal/d)

Source	DF	Sum of squares	Mean Square	P-value
Model	8	0.16880101	0.02110013	0.0005
Treatment	3	0.00428207	0.00142736	0.6501
Block	5	0.16166040	0.03233208	0.0001
Error	13	0.03307626	0.00254433	
Corrected Total	21	0.20187727		

Table B.33 Analysis of variance for the mean of ME intake (Mcal/kgDM)

Source	DF	Sum of squares	Mean Square	P-value
Model	8	0.02851740	0.00356468	<.0001
Treatment	3	0.02714695	0.00904898	<.0001
Block	5	0.00009528	0.00001906	0.9047
Error	13	0.00082805	0.00006370	
Corrected Total	21	0.02934545		

Table B.34 Analysis of variance for the mean of ruminal pH at 0 h post-feeding

Source	DF	Sum of squares	Mean Square	P-value
Model	8	0.22580087	0.02822511	0.1830
Treatment	3	0.04761905	0.01587302	0.4358
Block	5	0.19095238	0.03819048	0.1010
Error	13	0.21238095	0.01633700	
Corrected Total	21	0.43818182		

Table B.35 Analysis of variance for the mean of ruminal pH at 4 h post-feeding

Source	DF	Sum of squares	Mean Square	P-value
Model	8	0.11685403	0.01460675	0.2574
Treatment	3	0.02177827	0.00725942	0.5516
Block	5	0.07827827	0.01565565	0.2346
Error	13	0.12905506	0.00992731	
Corrected Total	21	0.24590909		

Table B.36 Analysis of variance for the mean of NH_3-N (mg/dl) at 0 h post-feeding

Source	DF	Sum of squares	Mean Square	P-value
Model	8	109.7508804	13.7188601	0.0229
Treatment	3	64.90656071	21.63552024	0.0118
Block	5	62.41896738	12.48379348	0.0439
Error	13	51.3669560	3.9513043	
Corrected Total	21	161.1178364		

Table B.37 Analysis of variance for the mean of NH_3-N (mg/dl) at 4 h post-feeding

Source	DF	Sum of squares	Mean Square	P-value
Model	8	97.4896116	12.1862015	0.0371
Treatment	3	14.79509494	4.93169831	0.3402
Block	5	82.20863161	16.44172632	0.0189
Error	13	52.3457884	4.0265991	
Corrected Total	21	149.8354000		

Table B.38 Analysis of variance for the mean BUN (mg/dl) at 0 h post-feeding

Source	DF	Sum of squares	Mean Square	P-value
Model	8	101.2728674	12.6591084	0.6171
Treatment	3	9.87389695	3.29129898	0.8900
Block	5	97.13696528	19.42739306	0.3539
Error	13	207.0262781	15.9250983	
Corrected Total	21	308.2991455		

Table B.39 Analysis of variance for the mean of BUN (mg/dl) at 4 h post-feeding

Source	DF	Sum of squares	Mean Square	P-value
Model	8	131.5863765	16.4482971	0.0519
Treatment	3	3.3522402	1.1174134	0.9041
Block	5	119.9727302	23.9945460	0.0205
Error	13	78.1688098	6.0129854	
Corrected Total	21	209.7551864		

Table B.40 Analysis of variance for the mean of total VFAs (mmol/l) at 0 h post-feeding

Source	DF	Sum of squares	Mean Square	P-value
Model	8	83.6510487	10.4563811	0.1649
Treatment	3	19.88620476	6.62873492	0.3676
Block	5	61.06963810	12.21392762	0.1290
Error	13	75.2440286	5.7880022	
Corrected Total	21	158.8950773		

Table B.41 Analysis of variance for the mean of total VFAs (mmol/l) at 4 h

Source	DF	Sum of squares	Mean Square	P-value
Model	8	299.6066220	37.4508277	0.0005
Treatment	3	194.9337068	64.9779023	0.0002
Block	5	88.9132302	17.7826460	0.0223
Error	13	59.3459098	4.5650700	
Corrected Total	21	358.9525318		

Table B.42 Analysis of variance for the mean of acetic acid (mol/100mol) at 0 h post-feeding

Source	DF	Sum of squares	Mean Square	P-value
Model	8	33.65024938	4.20628117	0.0623
Treatment	3	24.72715469	8.24238490	0.0154
Block	5	10.33224635	2.06644927	0.3349
Error	13	21.18958698	1.62996823	
Corrected Total	21	54.83983636		

Table B.43 Analysis of variance for the mean of acetic acid (mol/100mol) at 4 h post-feeding

Source	DF	Sum of squares	Mean Square	P-value
Model	8	11.72297936	1.46537242	0.9521
Treatment	3	4.45615208	1.48538403	0.8205
Block	5	5.75593542	1.15118708	0.9390
Error	13	63.07814792	4.85216522	
Corrected Total	21	74.80112727		

Table B.44 Analysis of variance for the mean of propionic acid (mol/100mol) at 0 h post-feeding

Source	DF	Sum of squares	Mean Square	P-value
Model	8	6.63691540	0.82961443	0.4108
Treatment	3	3.78607374	1.26202458	0.2154
Block	5	2.20758207	0.44151641	0.7039
Error	13	9.63223460	0.74094112	
Corrected Total	21	16.26915000		

Table B.45 Analysis of variance for the mean of propionic acid (mol/100mol) at 4 h post-feeding

Source	DF	Sum of squares	Mean Square	P-value
Model	8	10.03882814	1.25485352	0.6814
Treatment	3	3.73428571	1.24476190	0.5675
Block	5	5.26572238	1.05314448	0.7053
Error	13	23.04883095	1.77298700	
Corrected Total	21	33.08765909		

Table B.46 Analysis of variance for the mean of butyric acid (mol/100mol) at 0 h post-feeding

Source	DF	Sum of squares	Mean Square	P-value
Model	8	20.63494343	2.57936793	0.2148
Treatment	3	13.58811161	4.52937054	0.0802
Block	5	7.13289827	1.42657965	0.5161
Error	13	20.86763839	1.60520295	
Corrected Total	21	41.50258182		

Table B.47 Analysis of variance for the mean of butyric acid (mol/100mol) at 4 h post-feeding

Source	DF	Sum of squares	Mean Square	P-value
Model	8	16.41252619	2.05156577	0.7033
Treatment	3	8.12170952	2.70723651	0.4696
Block	5	5.19956952	1.03991390	0.8771
Error	13	39.30167381	3.02320568	
Corrected Total	21	55.71420000		

Table B.48 Analysis of variance for the mean of C2:C3 ratio at 0 h post-feeding

Source	DF	Sum of squares	Mean Square	P-value
Model	8	1.57772222	0.19721528	0.2148
Treatment	3	0.99406540	0.33135513	0.0889
Block	5	0.50973040	0.10194608	0.5504
Error	13	1.59565960	0.12274305	
Corrected Total	21	3.17338182		

Table B.49 Analysis of variance for the mean of C2:C3 ratio at 4 h post-feeding

Source	DF	Sum of squares	Mean Square	P-value
Model	8	1.49017208	0.18627151	0.7523
Treatment	3	0.52241905	0.17413968	0.6423
Block	5	0.85515238	0.17103048	0.7266
Error	13	3.94626429	0.30355879	
Corrected Total	21	5.43643636		

Table B.50 Analysis of variance for the mean of bacteria ($x10^{10}$ cell/ml) at 0 h post-feeding

Source	DF	Sum of squares	Mean Square	P-value
Model	8	1.73817222	0.21727153	0.0850
Treatment	3	1.01668207	0.33889402	0.0423
Block	5	0.61940707	0.12388141	0.3132
Error	13	1.21460960	0.09343151	
Corrected Total	21	2.95278182		

Table B.51 Analysis of variance for the mean of bacteria ($x10^{10}$ cell/ml) at 4 h post-feeding

Source	DF	Sum of squares	Mean Square	P-value
Model	8	4.50834188	0.56354274	0.0148
Treatment	3	0.96317976	0.32105992	0.1344
Block	5	3.34406643	0.66881329	0.0121
Error	13	1.87940357	0.14456951	
Corrected Total	21	6.38774545		

Table B.52 Analysis of variance for the mean of total protozoa (x10^6 cell/ml) at 0 h post-feeding

Source	DF	Sum of squares	Mean Square	P-value
Model	8	21.75162676	2.71895334	0.3369
Treatment	3	11.11677827	3.70559276	0.2099
Block	5	12.44052827	2.48810565	0.3784
Error	13	27.83530506	2.14117731	
Corrected Total	21	49.58693182		

Table B.53 Analysis of variance for the mean of total protozoa (x10^6 cell/ml) at 4 h post-feeding

Source	DF	Sum of squares	Mean Square	P-value
Model	8	15.18138799	1.89767350	0.0054
Treatment	3	8.80449405	2.93483135	0.0033
Block	5	6.73966071	1.34793214	0.0307
Error	13	4.95008929	0.38077610	
Corrected Total	21	20.13147727		

Table B.54 Analysis of variance for the mean of *Holotrich* sp (x10^5 cell/ml) at 0 h post-feeding

Source	DF	Sum of squares	Mean Square	P-value
Model	8	10.46570617	1.30821327	0.5467
Treatment	3	4.21949405	1.40649802	0.4395
Block	5	7.55282738	1.51056548	0.4381
Error	13	18.98883929	1.46067995	
Corrected Total	21	29.45454545		

Table B.55 Analysis of variance for the mean of *Holotrich* sp (x10^5 cell/ml) at 4 h post-feeding

Source	DF	Sum of squares	Mean Square	P-value
Model	8	102.0932934	12.7616617	0.1099
Treatment	3	66.42094494	22.14031498	0.0407
Block	5	32.02511161	6.40502232	0.4232
Error	13	78.2248884	6.0172991	
Corrected Total	21	180.3181818		

Table B.56 Analysis of variance for the mean of *Entodiniomorphs* sp (x10^6 cell/ml) at 0 h post-feeding

Source	DF	Sum of squares	Mean Square	P-value
Model	8	19.12895022	2.39111878	0.3281
Treatment	3	9.86273810	3.28757937	0.2018
Block	5	10.75607143	2.15121429	0.3792
Error	13	24.10434524	1.85418040	
Corrected Total	21	43.23329545		

Table B.57 Analysis of variance for the mean of *Entodiniomorphs* sp (x10^6 cell/ml) at 4 h post-feeding

Source	DF	Sum of squares	Mean Square	P-value
Model	8	8.83606686	1.10450836	0.0025
Treatment	3	4.81726004	1.60575335	0.0021
Block	5	4.44088504	0.88817701	0.0108
Error	13	2.42086496	0.18622038	
Corrected Total	21	11.25693182		

Table B.58 Analysis of variance for the mean of fungal zoospores ($x10^6$ cell/ml) at 0 h post-feeding

Source	DF	Sum of squares	Mean Square	P-value
Model	8	45.75350446	5.71918806	0.0009
Treatment	3	0.46892113	0.15630704	0.8944
Block	5	45.18925446	9.03785089	0.0002
Error	13	10.15149554	0.78088427	
Corrected Total	21	55.90500000		

Table B.59 Analysis of variance for the mean of fungal zoospores ($x10^6$ cell/ml) at 4 h post-feeding

Source	DF	Sum of squares	Mean Square	P-value
Model	8	89.9689010	11.2461126	0.0488
Treatment	3	5.66971540	1.88990513	0.7094
Block	5	82.07159040	16.41431808	0.0192
Error	13	52.4488263	4.0345251	
Corrected Total	21	142.4177273		

Table B.60 Analysis of variance for the mean of PCV at 0 h post-feeding

Source	DF	Sum of squares	Mean Square	P-value
Model	8	88.3628247	11.0453531	0.3388
Treatment	3	35.87797619	11.95932540	0.2955
Block	5	57.51130952	11.50226190	0.3161
Error	13	113.4553571	8.7273352	
Corrected Total	21	201.8181818		

Table B.61 Analysis of variance for the mean of PCV at 4 h post-feeding

Source	DF	Sum of squares	Mean Square	P-value
Model	8	24.1724161	3.0215520	0.9145
Treatment	3	5.72544643	1.90848214	0.8682
Block	5	19.44211310	3.88842262	0.7815
Error	13	104.1912202	8.0147092	
Corrected Total	21	128.3636364		

Appendix C. Calculation of production cost of rearing goat with TMR containing OPF silage supplemented with enzyme

1. Cost of live goat

= initial weight of goat (kg) x price (baht/kg live weight)

Table C.1 The cost of live goat

Level of enzyme (g/kgDM of TMR)	Average of initial weight (kg)	Price* (baht/kg live weight)	Total cost (baht/head)
0	13.90	100	1,390
2	13.64	100	1,364
4	13.24	100	1,324
6	14.00	100	1,400

*Price of goat (100 baht/kg) was based on the goat's price of Small Ruminant Research and Development Center, Faculty of Natural Resources, Prince of Songkla University.

2. Cost of feed

= cost of TMR + cost of enzyme

= {total TMR intake (kg) x price of TMR (baht/kg)} + {amount of enzyme (kg) x price of enzyme (baht/kg)}

Table C.2 Total feed cost

Level of enzyme (g/kgDM of TMR)	Total TMR intake (kg)	Total TMR intake (kgDM)	Total enzyme (g)	TMR price (baht/kg)	Enzyme price (baht/kg)	TMR cost (baht)	Enzyme cost (baht)	Total feed cost (baht/h)
0	40.30	22.57	0.00	3.98	150	160.40	0.00	160.40
2	39.35	22.04	17.63	3.98	150	156.21	2.64	159.26
4	37.99	21.28	34.04	3.98	150	151.21	5.11	156.31
6	39.55	22.15	53.15	3.98	150	157.39	7.97	165.36

3. Cost of mineral block

$$= \frac{\text{number of mineral block x mineral block price (kg/block)}}{\text{number of goat}}$$

Table C.3 Cost of mineral block

Level of enzyme (g/kgDM of TMR)	Number of mineral block	Price (baht/block)	Number of goat	Total cost (baht/head)
0	3	55	6	27.50
2	3	55	6	27.50
4	3	55	6	27.50
6	3	55	6	27.50

4. Cost of labor

$$= \frac{\text{rearing day x cost of labor (baht/day)}}{\text{number of goat}}$$

$$= \frac{90 \times (17 \times 3)}{24} = 191.25$$

Note: The salary of worker of small ruminant station was 4,100 baht/month. They work 8 hours/day with 3 hours for take care the goat. Hence, the cost of labor is 17 baht/hour. They spend 3 hour/day to take care goat.

5. Cost of deworming treatment

$$= \frac{\text{Price of ivermectin (baht/bottle)}}{\text{dosage (ml/50 kgBW) x quantity in 1 bottle (ml)}}$$

$$= \frac{1,150}{50\text{kg/1ml} \times 100 \text{ ml/bottle}}$$

= 0.23 baht/kg BW

Table C.4 Cost of deworming treatment

Level of enzyme (g/kgDM of TMR)	Average of initial weight (kg)	Price (baht/kg)	Total cost (baht/head)
0	13.90	0.23	3.20
2	13.64	0.23	3.14
4	13.24	0.23	3.05
6	14.00	0.23	3.22

6. Sale price of live goat

= final weight of goat (kg) x price (baht/kg live weight)

Table C.5 Total income of goat after rearing during 90 days.

Level of enzyme (g/kgDM of TMR)	Average of final weight (kg)	Price* (baht/kg)	Total revenue (baht/head)
0	16.86	130	2,192.67
2	17.28	130	2,225.60
4	16.29	130	2,085.33
6	16.63	130	2,162.33

*The sale price of goat (130 bahl/kg) is based on the price of central goat market of Satun province.

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List of Publication and Proceeding

1. Wahyuni, R. D., Ngampongsai, W., Wattanachant , C., Visessanguan, W. and Boonpayung, S. 2011. Effects of enzyme levels in total mixed ration containing oil palm frond silage on intake and growth performance of male goat. The 3rd International Conference on Sustainable Animal Agriculture for Developing Countries (SAADC) 2011, July 26-29, 2011; Nakhon Ratchasima, Thailand.

2. Wahyuni, R. D., Ngampongsai, W., Wattanachant , C., Visessanguan, W., and Boonpayung, S. 2012. Effects of enzyme levels in total mixed ration containing oil palm frond silage on intake, rumen fermentation, and growth performance of male goat. (Submitted to Songklanakarin Journal of Science and Technology on 16 November 2011).