Original Article

Effects of enzyme levels in total mixed ration containing oil palm frond silage on intake, rumen fermentation, and growth performance of male goat

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Abstract

This experiment was conducted to study the effects of supplementing the total mixed ration (TMR) containing oil palm frond (OPF) silage with different levels of enzyme on feed intake and growth performance of goat. Twenty four post-weaning Boer × Thai Native crossbred male goats with initial body weight (BW) of 11-18 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. The enzyme mixture produced by *Aspergillus* spp. BCC 274, containing approximately 1×10^7, 9×10^6, 2×10^6, 1×10^6 and 2×10^6 unit/kg dry weight for xylanase, β-glucanase, cellulase, mannanase and amylase, respectively, was supplemented to the concentrate portion at 0, 2, 4 and 6 g/kgDM of the TMR. The results showed that the supplementation of enzyme to the TMR did not affect (P>0.05) dry matter intake (DMI). Goats receiving TMR supplemented with enzyme at 2 g/kgDM tended to have higher ADG and better feed per gain ratio as compared with other treatments. Coefficient of DM digestibility of TMR was not significantly affected by the enzyme supplementation. In addition, there were no significant differences (P>0.05) among treatments regarding, average NH_3-N concentration, the amount of C_2, C_3 and C_4 in the rumen fluid and BUN concentration. However, overall mean of ruminal NH_3-N concentration was significantly lower in goat receiving TMR supplemented with enzyme at 2 g/kgDM than that of goat receiving TMR with no enzyme supplementation (P<0.05). 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.

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

1. Introduction

Oil palm frond (OPF) is one of the by-products that are abundantly produced and has potential as a source of roughage for ruminants (Dahlan *et al.*, 2000; Kawamoto *et al.*,

2001). A number of processing techniques have developed to improve feeding values of OPF. Serving OPF silage together with concentrate in total mixed ration (TMR) is suggested to improve their palatability or intake (Dahlan *et al.*, 2000). In recent years, studies using exogenous enzyme additive in ruminant diet have reported improvement in digestibility and animal performance. Beauchemin *et al.* (2003) suggested that applying exogenous fibrolytic enzyme in ruminant diet improved feed digestibility but the result was varied. Some

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positive effects of 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.

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 fibrolytic enzyme containing endoglucanase and xylanase activities to the rumen of sheep fed a mixed grass hay: concentrate (70:30 DM basis) diet and observed no effects on diet digestibility. However, enzyme supplementation increased in situ DM disappearance of grass hay. There was also greater NDF disappearance in enzyme fed sheep compared with unsupplemental animals. The results indicated that supplementing enzyme directly into the rumen increased the fibrolytic activity in the 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 effect on feed intake or birth weight, but enzyme supplementation increased the weaning weight and milk production, and improved milk composition. The results suggested that improvement of milk production without apparent change in feed intake was through improved feed utilization. However, the information on the effects of enzymes on ruminal fermentation in small ruminants is limited. Therefore, the objective of this study was to determine the effect of supplementing the TMR containing OPF silage with different levels of enzyme on feed intake, rumen fermentation, and growth performance of goat.

2. Materials and Methods

2.1 OPF silage preparation

The fresh OPF gathered from Thepa Research and Training Station, Faculty of Natural Resources, Prince of Songkla University, was chopped to 1-2 cm length and blended uniformly. A portion 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.

2.2 Enzyme mixture

The enzyme product used in this study was a noncommercial product derived from *Aspergillus* spp. BCC 274, compliant with the current specifications for food-grade enzyme and generally recognized as safe. The enzyme activities measured at pH 6.8 were 1×10^7, 9×10^6, 2×10^6, 1×10^6 and 2×10^6 unit/kg dry weight for xylanase, β-glucanase, cellulase, mannanase and amylase, respectively.

2.3 TMR preparation

The TMR used in the study contained, in grams per kilogram (DM basis) the following: oil palm frond silage, 600; broken rice, 148; ground corn, 125; soybean meal, 57; fish meal, 50; urea, 10 and minerals, 10 (Table 1). The concentrate was prepared as a loose mix then the enzyme was added to the concentrate portion before compositing with OPF silage. By calculation, the TMR contained approximately 15% of crude protein and 60% of total digestible nutrient (TDN) that meets the nutrient requirements for goat according to NRC (1985). The TMR was prepared daily.

2.4 Animals and experimental procedure

Feeding trial experiment was conducted using twenty four post-weaning Boer x Thai Native crossbred male goat, at the age of 9-12 months with an initial weight of 11-18 kg. All goats were drenched for internal worms (Invermectin, IDECTIN®, The British Dispensary, Co., Ltd.) prior to commencing the experiment. Each goat was placed individual pen where water and mineral salt were available at all times. The study 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 the enzyme levels supplementation (0, 2, 4, and 6 g/kgDM of TMR). 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 and voluntary feed intake (VFI) was determined. Fresh water was avail-

Table 1. Composition of ingredient of the TMR (%DM basis)

Item	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
TDN (%)	60
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.

able 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°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°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 the sample collection period, rumen fluid sample was collected at 0 and 4 hours post feeding, using a stomach tube connected with a vacuum pump. The rumen fluid samples were strained through two cheesecloths and 3 ml of H_2SO_4 (1 M) were added to 30 ml of rumen fluid. The mixture was centrifuged at 16,000 X g for 15 minutes and supernatant was stored at -20°C prior to ammonia nitrogen (NH_3-N) and volatile fatty acid (VFA) analysis. Blood samples were collected via the jugular vein into a plastic tube at the same time of rumen fluid sampling for blood urea nitrogen (BUN) analysis. The goat was weighted every two weeks before feeding in the morning during the data collection period for daily gain and feed conversion ratio calculation.

2.5 Laboratory analyses

Feeds, refusal and feces were chemically analyzed using proximate analysis according AOAC (1990) procedure. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined with the procedure of Goering and Van Soest (1970). AIA in feed and feces were analyzed and nutrient apparent digestibility coefficient was calculated using the method described by Van Keulen and Young (1977). Ruminal NH_3-N was determined using the Kjeldahl method (AOAC, 1990) and VFA analyses using a gas chromatography (GC 6890, Agilent Technologies) according to Josefa *et al.* (1999). BUN was measured using diagnostic kits (Stanbio Urea Nitrogen Liqui-UV®, Stanbio Laboratory).

2.6 Statistical analysis

All data were 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.

3. Results and Discussion

The chemical composition of OPF silage used in this study is shown in Table 2. OPF silage contained 4.12%CP, 76.19% and 58.40%ADF (DM basis). 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%. By contrast, 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. The TMR used in the present study contained OPF silage: concentrate ratio of 60:40. This high forage diet was used in order to evaluate the effects of the enzyme on fiber digestion. The TMR supplemented with different levels of enzyme has similar DM, OM, Ash, CP, EE, ADF and ADL contents which averaged 96.01, 91.70, 8.31, 14.83, 1.93, 36.5 and 11.05%, respectively (Table 3). Slightly lower concentration of CP 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 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 the concentrate portion. The low NDF content of the TMR supplemented with enzyme might be due to the enzyme increasing the susceptibility of the diet to the detergents used in fiber

Table 2. 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 3. Chemical composition of TMR supplemented with different level of enzyme (%DM basis)

Items[1]	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
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

[1] DM= Dry matter, OM= Organic matter, CP= Crude protein, EE= Ether extract, NDF = Neutral detergent fiber, ADF= Acid detergent fiber, ADL= Acid detergent lignin

analysis (Krause *et al.*, 1998). Contrastly, Hristov *et al.* (1998) 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 a result of enzymatic solubilization of plant fibers. Whether the decreasing of 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 biological effects of the chemical composition of the TMR (Beauchemin *et al.*, 2000;

Kung *et al.*, 2000; 2002). The ADL content in each ration was relatively high and it might be a limitation in term of feed intake and digestibility (Hart and Wanapat, 1992; Van Soest, 1994). Chanjula *et al.* (2007) reported that the goat has a limited rumen capacity to use highly lignified feed.

Table 4 illustrated the effect of enzyme supplementation in TMR on feed intake, growth performance and nutrient digestibility of goat. There was no significant difference (P>0.05) among treatments regarding dry matter intake (DMI). The effects of enzyme supplementation on average daily gain (ADG) was not statistically significant (P>0.05) but goats receiving TMR supplemented with enzyme at 2 g/kgDM had numerically higher ADG than the goats receiving TMR supplemented with enzyme at 0, 4, 6 g/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.

In the current study, the DMI of goat ranged from 2.83-2.88 %BW, which was less than the requirement for goat in tropical regions, reported to be 3.0-3.1 %BW (Devendra and McLeroy, 1982) and 3.05-3.66 %BW (Ashok and Wadhwani, 1992). Furthermore, AFRC (1998) recommended that the level of DMI for growing goats was 66 $g/kgBW^{0.75}$. Allison (1985), cited by Kawas *et al.* (1999), reported that the low nutrient intake was the most important factor limiting animal performance. Thus the weight gain and ADG of the goat in the current study was lower than the target ADG (50 g/d). It is possible that low DMI could have been attributed to a high ADL with low fermentation rate and digestibility leading to a low rate of disappearance through digestion or

Table 4. Effect of enzyme supplementation in TMR on feed intake, growth performance and digestibility of goat

Items	Level of enzyme (g/kgDM of TMR)				SEM*	P-value
	0	2	4	6		
Body weight/BW (kg)						
Initial body weight	13.90	13.60	13.66	14.00	0.14	0.2176
Final body weight	16.86	17.28	16.29	16.63	0.25	0.1369
Total weight gain	2.97	3.67	2.63	2.63	0.26	0.0719
ADG (g/d)	32.96	40.86	29.27	29.26	2.92	0.0792
DM intake						
g/h/d	442.89	437.26	422.46	434.57	10.99	0.6739
%BW	2.88	2.83	2.84	2.86	0.06	0.9281
$g/kgBW^{0.75}/d$	57.04	56.04	55.64	56.48	1.29	0.8979
Feed per gain	14.36	10.76	14.79	17.02	1.33	0.0564
Digestibility (%)						
DM	47.40	46.50	50.82	47.11	1.68	0.3984
OM	51.09	49.80	53.54	51.29	1.83	0.6485
CP	52.13	53.81	57.02	53.41	1.83	0.3842

*SEM: standard error of the means

passage and limited feed intake (Hart and Wanapat, 1992; Wanapat, 2000).

In the present experiment, apparent digestibility of DM, OM and CP, however, were not affected by the supplementation of enzyme. Exogenous fibrolytic enzyme has typically been observed to increase the initial rate but not the extent of DM digestion when used in ruminant diets (Feng *et al.*, 1996; Wang and McAllister, 2002). Moreover ADG and DMI tended to decrease when the levels of enzyme supplementation in TMR were increased. Gilardo *et al.* (2008) declared that the 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. Although an increase of digestibility occurred, the improvement of animal performance depended on the physiological status of the animal and the condition during the experiment. 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. Animal response was greatest when fiber digestion was compromised and when energy was the first-limiting nutrient in the ration.

Rumen fermentation parameters were measured for pH, NH_3-N and volatile fatty acid (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 post-feeding and 4 h and overall means are given in Table 5.

Ruminal NH_3-N concentration at 4 h post feeding was similar among treatments, ranging from 12.69 -15.05 mg/dl, except at 0 h post feeding, and overall means were affected ($P<0.05$) by treatments, ranging from 12.43-16.90 and 12.56-15.86 mg/dl, respectively, and were significantly decreased by enzyme supplementation at 2 g/kgDM. Ruminal NH_3-N concentration was 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 this study was higher than 5-8 mg/dl, which is the optimal level of NH_3-N for microbial protein synthesis (Satter and Slyter, 1974). Likewise, BUN concentration at 0 h post-feeding and 4 h and overall means were similar among treatments, ranging from 27.10-29.04 mg/dl, 30.88-33.32 mg/dl and 29.13-31.02 mg/dl, respectively. The BUN prior to morning feeding of the goats tended to be lower than that at 4 h. The results agreed with Eggum (1970) who reported that urea content in the blood reached

a maximum 3 h after feeding. Observed BUN concentration was close to the optimal level in normal goats, which was been reported to be in the range of 11.2-27.7 mg/dl (Lloyd, 1982).

The effects of enzyme supplementation on production of total VFAs concentration, acetic acid, propionic acid and butyric acid proportion are shown in Table 5. Overall means of total VFAs, acetate, propionate and butyrate in the rumen were not affected by dietary treatments. However, the concentration of total VFAs at 4 h was significantly higher for goats fed TMR without enzyme supplementation. The amount of total VFAs reflected the fermentation activity in the rumen, higher total VFAs means more rumen fermentation activity (Abdullah *et al.*, 1995). The proportions of acetate, propionate and butyrate among treatments were similar ($P>0.05$), except the proportion of acetate 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) showing that the proportion of acetate was higher for cows fed the low level of enzyme (Natugrain 33-L; BASF corporation Ludwigshafen, Germany) compared with the control (no enzyme). Boonthep *et al.* (2011) reported that there were significant differences in fermentable soluble fraction between untreated and enzyme treated TMR containing OPF silage. They reported that the increase of fermentable soluble fraction and potential of extent of gas production with enzyme treated TMR indicated 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 current study that ruminal NH_3-N concentration was decreased with a low level of enzyme supplementation (Table 5) due to an increase in ruminal availability of slowly digestible carbohydrate. The acetate to propionate ratio tended to be slightly higher by inclusion of enzyme in the diets, the enzyme supplementation increased the daily output of acetate without decreasing the production of propionate. The ratio of propionate, butyrate, and acetate in this study was, however, in accordance with the reports by Bowen (2010) that the molar ratio of acetic: propionic: butyric is roughly 70:20:10.

Lastly, goats receiving TMR supplemented with enzyme at 2 g/kgDM showed the best performance. Perhaps, this level of enzyme provided sufficient contact between enzyme and fiber as a substrate. Nsereko *et al.* (2002) declared that exogenous enzyme stimulated an increase in microbial population, which increased digestibility and animal performance. On the other hand, increasing the level of enzyme was followed by a reduction in performance of the goat. They also considered that the supplementation of low level of enzyme to ruminant feed caused beneficial disruption of the surface structure of feed both before and after ingestion. When excess enzyme was applied, the beneficial breakdown of the feed surface may be minimized due to the enzyme attached to feed restricting microbial attachment and causing limited digestion of feed.

Table 5. Effect of enzyme supplementation in TMR on ruminal fermentation, BUN, and VFA proportion

Items	Level of enzyme (g/kgDM of TMR)				SEM[1]	P-value
	0	2	4	6		
NH$_3$-N (mg/dl)						
0 hr, post-feeding	16.67[a]	12.43[b]	15.79[ab]	16.90[a]	0.81	0.0118*
4 hr	15.05	12.69	13.92	13.58	0.82	0.3402
Mean	15.83[a]	12.56[b]	14.86[a]	15.24[a]	0.94	0.0165*
BUN (mg/dl)						
0 hr, post-feeding	29.04	27.10	27.38	28.48	1.60	0.8331
4 hr	33.01	33.32	30.88	32.14	0.95	0.3957
Mean	30.72	30.08	29.67	30.16	1.60	0.9419
Total VFA (mmol/L)						
0 hr, post-feeding	25.83	23.28	24.14	25.10	0.98	0.3676
4 hr	41.20[a]	33.59[b]	35.35[b]	34.88[b]	0.87	0.0002**
Mean	33.52	28.44	29.74	29.99	2.86	0.3734
Acetate (mol/100mol)						
0 hr, post-feeding	72.07[b]	73.98[a]	74.62[a]	74.57[a]	0.52	0.0154*
4 hr	73.17	73.07	72.52	73.80	0.89	0.8205
Mean	72.62	73.52	73.56	74.18	0.70	0.1894
Propionate (mol/100mol)						
0 hr, post-feeding	14.84	14.67	13.71	14.30	0.35	0.2154
4 hr	15.17	15.46	14.40	14.56	0.54	0.5675
Mean	15.01	15.06	14.06	14.43	0.42	0.1146
Butyrate (mol/100mol)						
0 hr, post-feeding	13.07	11.34	11.66	11.12	0.51	0.0802
4 hr	11.65	11.46	13.07	11.63	0.70	0.4696
Mean	12.36	11.40	12.36	11.38	0.59	0.2105
Acetate: Propionate ratio						
0 hr, post-feeding	4.87	5.06	5.46	5.22	0.14	0.0889
4 hr	4.83	4.76	5.08	5.13	0.22	0.6423
Mean	4.85	4.91	5.27	5.18	0.17	0.0859

[1] SEM: standard error of the means
* significant (P<0.05)
** significant (P<0.01)
Different superscripts (a-b) in a row are significantly different.

4. Conclusion

The result of this experiment indicated that the application of fibrolytic enzyme produced by *Aspergillus* spp. BCC 274 at low level (2 g/kgDM) to the concentrate portion of TMR containing OPF silage could increase ruminal availability of slowly digestible carbohydrate and improve goat performance.

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