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Effect of fibrolytic enzyme supplementation on voluntary feed intake, nutrient digestibility and nitrogen utilization in meat goats

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ABSTRACT: Twelve male 75%Boer and 25%Anglonubian goats with initial body weight of 25 $\pm$ 2.5 kg, were used in a completely randomized design. Animals were received either FTMR with or without enzyme supplementation from tomato pomace treated with *Aspergillus niger*. The experiment was lasted for 90 days. The experiment revealed that goats fed FTMR with enzyme was higher (P<0.05) in voluntary feed intake when expressed as gDM/d than goats fed FTMR alone. Average daily gain (ADG) and feed conversion ratio (FCR) were higher (P<0.05) in goats fed FTMR than goats fed control. Digestibility of dry matter, organic matter, crude protein, neutral detergent fiber and acid detergent fiber were greater (P<0.05) in goats fed E-FTMR than in goats fed FTMR. Total volatile fatty acids, acetate, propionate and butyrate concentrations at 4 hours post feeding were higher (P<0.05) in goats fed E-FTMR than goats fed FTMR. Nitrogen absorption and nitrogen retention when expressed as % of nitrogen intake were greater (P<0.05) in goats fed E-FTMR than goats fed FTMR. Based on the experimental data, it can be concluded that supplemental enzyme from dried tomato pomace treated by *Aspergillus niger* did increase feed intake, nutrient digestibility and nitrogen utilization in meat goats.

Keywords: *Aspergillus niger*; tomato pomace; enzyme; voluntary feed intake; digestibility; nitrogen utilization

Introduction

Agricultural and industrial by-products are well accepted by many countries to use as ruminant feeds such as rice straw, sugarcane bagasse, sugarcane tops, corn stover, oil flam fronds, dried tomato pomace, dried citric acid residues (Abid et al., 2019; Wongnen et al., 2009). The limitations of agricultural-industrial by-products are relied on their nutritive values and digestion. Recently, biotechnology especially enzymatic technology has been exclusively developed and its price is reasonable to apply in animal ration. Enzyme supplementation is a novel method in ruminant nutrition in contrast with non-ruminants, which enzyme application has been extensively incorporated into feed formulation. While ruminant nutrition, enzyme has been recently increasing interested in addition in diet to enhance non-fiber and fiber fractions. Fibrolytic enzyme is an exogenous enzyme, which has been intensively investigated to improve fiber digestibility of forages. Beuchemin et al., 2003) reported that fibrolytic enzyme supplementation enhanced fiber digestibility of forage diet. In accordance with Krause et al. (1998) revealed that inclusion of enzyme elevated ADF digestibility when included into a high concentrate diet. Moreover, Colombatto et al. (2003) showed that supplemental enzyme increased NDF digestibility. Following by the reported of Shekhar et al. (2010) who demonstrated that fibrolytic enzyme supplementation enhanced milk production in dairy buffalo. In agreement with the reported by Hristov et al. (2000) found that fibrolytic enzyme supplementation increases ruminal and intestinal nutrient digestibility. Tang et al. (2008) studied the effect of yeast culture and fibrolytic enzyme supplementation improves in vitro gas production of cereal straw. Khanh et al. (2012) found that fibrolytic enzyme supplementation in fermented total mixed ration (FTMR) did not improve fiber digestion in dairy cows but fibrolytic enzyme supplementation in total mixed ration (TMR) did increase fiber digestion. Abid et al. (2019) concluded that exogenous fibrolytic enzyme could enhance the use of low-quality agro-industrial by-products.

Thus, the current study aims to investigate the effect of exogenous fibrolytic enzyme (xylanase and cellulase) supplementation on voluntary feed intake, nutrient digestibility, rumen fermentation end products and nitrogen utilization in meat goats.

Materials and methods

This study was carried out at Nong Raweing Education Center, Rajamangala University of Technology Isan, Nakhon Ratchasima, Thailand. The experimental period was extended from November 2018 – April 2019. This experimental research has been conducted according to the experimental rules of National Research Council and approved by the ethical committee of Rajamangala University of Technology Isan.

Animals and Diets

Twelve male 75%Boer and 25%Anglonubian goats with initial body weight of 25 $\pm$ 2.5 kg, were used in a completely randomized design. Goats were received either FTMR with or without enzyme supplementation. Rice straw was used as main roughage source in FTMR (Table 1). Goats were housed in individual wooden pens and animals were adjusted to the same diet for period of 14 days before dietary treatments were applied to animals. Goats were fed ad libitum and clean water was supplied at all time. The experiment was lasted for 90 days.

Preparation of enzymes from dried tomato pomace

The fibrolytic enzymes were prepared according to Saithi et al. (2016). The solid-state fermentation was used to prepare the fungal culture with tomato pomace used as substrate. The culture of *Aspergillus niger* was purchased from the National Center for Genetic Engineering and Biotechnology (BIOTEC, Pathum Thani, Bangkok, Thailand). After processing completion, substrate was dried at 50 C for 3-5 days before fibrolytic enzyme was applied for the experiment. The activities of xylanase and cellulase from dried tomato pomace treated with A. niger were 37.815 x 10^6 and 60.998 x 10^6 units per kg dry mater, respectively.

Fermented total mixed ration making

Table 1 was demonstrated the ingredients of diets and these ingredients were thoroughly mixed and the mixtures were adjusted for moisture content at about 50% by added water. And then mixtures were filled in the recycled plastic bucket (Yuangkang et al., 2004; Wongnen et al., 2010). The mixtures were anaerobically fermented for period of 21 days before experiment commencement.

Sample collection

Voluntary feed intake was daily recorded and feed residue samples were determined. Body weight was monthly weighed for 2 days consecutively. During the last 7 days of the experiment, feces and urine samples were quantitatively collected as described by Yuangklang et al. (2010). The macronutrient composition of the feeds and feces were determined by the same methods. The ash content was determined by combustion at 550 °C for 16 h (AOAC, 2010) and the nitrogen (N) contents were determined by the micro Kjeldahl method (AOAC, 2010). A factor of 6.25 was used to convert N into crude protein (CP). The neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents were determined according to the method of Van Soest et al. (1991). Urine samples were analyzed for nitrogen (AOAC, 2010).

Rumen samples were collected through the stomach tube with connecting with vacuum pump at 0 and 4 h post morning feeding. The rumen fluid samples were strained through four cheesecloths and immediately rumen pH was measured using a portable pH meter (Hanna Instruments HI8424 microcomputer) (Kallang Way, Singapore). Then, rumen samples were added with 1 ml of $H_2 SO_4$ (1 M) into 10 ml of rumen fluid samples. The rumen samples were used for NH_3-N analysis using the micro-Kjeldahl method (AOAC, 2010). On the same occasion of rumen sampling, blood samples were collected via the jugular vein into a plastic tube for blood urea nitrogen (BUN) according to Bromner and Keeny (1967).

Statistical Analysis

Data were statistically analyzed by Student's t-test using SPSS (1998) for windows 9.0 (SPSS Inc., Chicago, IL, USA). The level of significance was pre-set at $P < 0.05$.

Results and discussions

Ingredients and chemical compositions of experimental diets are shown in **Table 1**. Fermented total mixed ration was formulated to contain 14%CP and 68%TDN (NRC, 1981). Rice straw was main roughage source due to it is abundant agricultural by-product in this region. Fermented total mixed ration is one of the most practical methods to improve total mixed ration containing low quality roughage. It clearly demonstrated that fermented total mixed ration did improve feed intake and nutrient digestibility in ruminants (Yuangklang et al., 2006; Wongnen et al., 2009).

Voluntary feed intake, average daily gain and feed conversion ratio

Voluntary feed intakes of goats fed FTMR with or without fibrolytic enzyme supplementation are shown in **Table 2**. Voluntary feed intake when expressed as gDM/d was significantly different (P<0.05) between treatments, but when expressed as %BW and g/kgBW$^{0.75}$ were not significantly different (P>0.05) between treatments. Similar result with Lee et al. (2014) reported that enzyme supplementation did not alter feed intake in Korean native goats. In accordance with reported by Wahyuni et al. (2012) declared that increasing enzyme levels did not influence feed intake of goats fed oil palm frond silage. In the current study, the DMI of goat ranged from 2.77-2.87 %BW, which similar with Wahyuni et al. (2012), which reported the feed intake when expressed as %BW was ranged from 2.83-2.88%BW. In addition, when expressed feed intake as g/kgBW$^{0.75}$ was ranged from 62.28-65.45 g/kgBW$^{0.75}$, which nearly result with AFRC (1998) recommended that dry matter intake was set at 66 g/kgBW$^{0.75}$ for growing goats. Average daily gain was higher (P<0.05) in goats fed FTMR with enzyme supplementation than in goats fed FTMR alone. This ADG finding was associated with increased nutrient digestibility in goats fed FTMR enriched with enzyme. Moreover, ADG was also related with nitrogen retention, more nitrogen retention implied that more protein synthesis for muscle development. Nsereko et al. (2002) speculated that exogenous enzyme stimulated an increase in microbial population, which increased digestibility and animal performance. Improved feed conversion ratio in goats fed FTMR enriched with enzyme goes hand in hand with increased feed intake and enhanced digestibility.

Table 1 Ingredients of fermented total mixed ration

Ingredients	Fermented total mixed ration (FTMR) kg/100kg	
	- Fibrolytic enzyme	+ Fibrolytic enzyme
Rice straw	20.0	20.0
Cassava chip	41.5	41.1
Soybean meal	17.0	17.0
Rice bran	10.5	10.5
Molasses	8.0	8.0
Urea	2.0	2.0
Salt	0.5	0.5
Dicalcium phosphate	0.3	0.3
Sulfur	0.1	0.1
trace minerals	0.1	0.1
Enzyme	-	0.4
Chemical composition, %		
Dry matter	63.46	63.55
	................% dry matter..................	
Ash	12.23	12.65
Organic matter	87.77	87.35
Crude protein	14.13	14.10
Ether extract	3.16	3.13
NDF	31.85	31.62
ADF	22.25	22.31
NFC	38.63	38.50
Total carbohydrate	70.48	70.12
TDN*	68.88	68.67
pH	5.36	5.20

NDF = neutral detergent fiber; ADF = acid detergent fiber; NFC = non-fiber carbohydrate; TDN = total digestible nutrients; Total carbohydrate = 100- crude protein-ether extract-ash; %NFC = 100 – (%NDF + %CP + %Fat + Ash)

*By calculation

Table 2 Effects on voluntary feed intake, nutrient digestibility of goats fed either FTMR with or without fibrolytic enzyme supplementation

Items	FTMR[1]	E-FTMR	SEM	P-value
Voluntary feed intake,				
gDM/d	665.87	689.36	7.15	<0.01
%BW	2.77	2.87	0.07	0.06
g/kg $BW^{0.75}$	62.28	65.45	1.32	0.459
Initial BW, kg	25.50	25.75	0.25	0.569
Final BW, kg	33.55	34.55	0.29	0.31
ADG, g/d	89.44	97.78	0.21	0.04
FCR	7.44	7.05	0.03	0.03
Digestibility, % of intake				
[1]DM	68.27[b]	73.34[a]	0.22	0.04
OM	71.81[b]	75.26[a]	0.25	0.04
EE	90.50	91.30	0.17	0.16
NDF	55.37 [b]	59.76[a]	0.22	0.02
ADF	45.96[b]	51.68[a]	0.23	0.02

[1]FTMR = fermented total mixed ration; E-FTMR = enzyme supplemented in FTMR; SEM =standard error the means; DM = dry matter; OM =organic matter; EE = ether extract; NDF = neutral detergent fiber; ADF = acid detergent fiber

Significantly difference was set at P<0.05

Table 3 Effect on rumen fermentation and blood urea nitrogen (BUN) of goats fed either FTMR with or without fibrolytic enzyme supplementation

Items	FTMR	E-FTMR	SEM	P-value
Total VFAs, mM				
0 hr	77.33	78.21	0.19	0.115
4 hr	80.27	85.43	0.23	0.004
Acetate, %				
0 hr	66.63	67.21	0.19	0.115
4 hr	69.27[b]	71.43[a]	0.23	0.004
Propionate, %				
0 hr	16.75	17.15	0.24	0.216
4 hr	19.35[b]	21.77[a]	0.27	0.003
Butyrate, %				
0 hr	16.62	15.64	0.31	0.09
4 hr	11.38[a]	6.80[b]	0.25	0.003
pH				
0 hr	6.84	6.78	0.157	0.168
4 hr	6.56	6.43	0.290	0.171
[1]NH_3-N in rumen, mg%				
0 hr	11.43	11.12	0.290	0.136
4 hr	12.50	12.21	0.277	0.108
BUN, mg%				

0 hr	11.23	11.19	0.25	0.164
4 hr	14.45	14.22	0.29	0.103

[1]FTMR = fermented total mixed ration; E-FTMR = enzyme supplemented in FTMR; NH_3-N = ammonia nitrogen in rumen; BUN = Blood Urea nitrogen, SEM=standard error of the mean

*Significantly difference was set at P<0.05

Total volatile fatty acids at 4 hours post morning feeding had higher (P<0.05) in goats fed in goats fed FTMR with enzyme supplementation than in goats fed FTMR alone. This would be a logical consequence of the increasing in voluntary feed intake and organic matter digestibility. Fermented organic matter in rumen by microbial activity produced both organic acids and ammonia nitrogen, which are primarily nutrients for microbes and animals (Hungate, 1966). Song et al. (2018) claimed that enzyme supplementation increased total volatile fatty acid, acetate and propionate concentrations, but did not alter butyrate concentration in goats compared with control group.

The concentration of ammonia nitrogen in the rumen (NH_3-N) was not significantly different between treatments (P>0.05). In rumen, the ammonia nitrogen is derived from break down of protein content which nitrogen is used by ruminal microorganisms in associated with keto acids derived from carbohydrate degradation. This current experiment at 4 hours post morning feeding, rumen ammonia was decreased. This might be partly explained by the rumen microbes used ammonia together with organic acids from carbohydrate degradation to create microbial mass, leading to increase nitrogen retention and improved growth rate. The optimal level of rumen NH_3-N for digestibility and intake was range from 15-20 mg% (Perdok and Leng, 1990). In contrast with Wahyuni et al. (2012) reported that increased enzyme level decreased NH_3-N concentration after 4 hours post feeding. Moreover, Wanapat (1999) and Wanapat et al. (2000) reported that when NH_3 level raised, from 1.7 to 5.6 mg%, total bacterial count, digestibilities of DM, NDF and ADF increased.

Nitrogen utilization

Table 4 illustrated the nitrogen utilization in goats fed FTMR with or without fibrolytic enzyme supplementation. Nitrogen intake, N in feces and N in urine in goats fed FTMR was not significantly differ (P>0.05) with goats fed TMR with enzyme, but N absorbed and N retained of goats fed FTMR with enzyme was higher (P<0.05) than in goats fed FTMR alone. It might be partly explained that supplementation of enzyme releases protein from feedstuffs and then rumen proteolytic bacteria can be easily degraded polypeptides (protein) into peptides and ammonia nitrogen eventually. The beneficial of increased retained N in goats fed FTMR with enzyme supplementation is associated with increasing productive performance. Similar resulted with Zhang et al. (2020) reported dairy cows fed fermented total mixed ration improved nitrogen utilization in dairy cows when compared with dairy cows fed total mixed ration.

Table 4 Effects on nitrogen utilization of goats fed either FTMR with or without fibrolytic enzyme supplementation

Items	FTMR	E-FTMR	SEM	P-value
N intake, g/d	17.17	17.67	0.25	0.224
N in feces, g/d	4.79	4.11	0.18	0.253
N in urine, g/d	1.53	1.55	0.06	0.877
N absorbed, g/d	12.38	12.46	0.32	0.27
N retained, g/d	10.85	10.91	0.33	0.22
N absorption, % intake	72.10[b]	76.74[a]	0.13	0.04
N retention, % intake	63.19[b]	67.97[a]	0.17	0.04

[1]FTMR = fermented total mixed ration; E-FTMR = enzyme supplemented in FTMR; SEM=standard error of the mean

Significantly difference was set at P<0.05

Conclusion

In conclusion, enzymes from fermented dried tomato pomace with *Aspergillus niger* added in FTMR for goats enhanced growth rate, feed efficiency and nitrogen utilization in goats. It was showed for the first time that enzyme derived from fermented tomato pomace added in fermented total mixed ration with rice straw as main roughage source improved growth performance and nitrogen utilization in goats.

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