

IN VIVO GREENHOUSE GASES EMISSIONS, NUTRIENT INTAKE, DIGESTIBILITY AND RUMEN PARAMETERS OF GROWING SHEEP AFFECTED BY DIETARY SILAGE SOURCES

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ABSTRACT

This experiment was done to evaluate *in vivo* greenhouse gases production, nutrient utilization and rumen parameters affected by silages in diets of sheep from Feb to May, 2018 at Can Tho University farm. Four male sheep with average live weight of 17,3±1,48 kg were arranged in a 4x4 Latin square design with 4 treatments including (1) *Paspalummatratum* (PA), and (2) 40% *Pennisetumpurpureum* silage (PPS40), (3) 40% *Operculinaturpethum* silage (OTS40) and (4) 40% *Psophocarpusscandens* silage (PSS40) replacing *Paspalummatratum* (%DM) in the diets. Methane and carbon dioxide were measured by using the chambers for 2 continuous days and analyzed by a Greenhouse Gas Analyser (USA). The results showed that the CH₄ production (g/kg OM intake) of sheep was significantly different among the treatments (P<0.05). The CH₄ production (g/kg OM intake) of the OTS40 treatment (6.37) was the lowest, following by the PSS40 treatment (6.93), the PPS40 treatment (7.69), and the PA treatment (9.60). The metabolizable energy (ME) intake was improved by the silage diets (P=0.056), while digestible protein intake and nitrogen retention of the OTS40, PSS40 PPS40 treatments tended to be higher than that of the PA treatment (P>0.05). Daily weight gains (g/day) of sheep were likely to be higher (P>0.05) for the OTS40, PPS40 and PSS40 treatments. The conclusion was that replacing *Paspalummatratum* grass by PPS, OTS or PSS at level of 40% (DM) in diets reduced CH₄ output, tended to improve total DM intake, crude protein digestibility, rumen parameters and nitrogen retention and reduced CH₄ output of growing sheep.

Keywords: methane, nitrogen retention, nutrient digestibility, sheep, silage.

INTRODUCTION

Greenhouse gas (GHG) emissions is one of the environmental problems for livestock production in recent years. While methane is a main source of GHG emissions from the agricultural sector. Methane has 23 times higher potential to exacerbate global warming than carbon dioxide. Livestock sector contributed about 14.5% of total global GHG emissions from human activities in 2010, while small ruminant production of meat and milk is responsible for 428.8 million tones CO₂. Sheep production produced 254.4 million tones CO₂ while 174.5 tones CO₂ (41 percent) for goat production (Anna, 2014). Although sheep population in Vietnam is small, it has exposed an increasing tendency. Sheep, namely Phan Rang sheep, are mainly raised in the South Central coast of Vietnam. This breed adapts well to the dry and hot climate and low quality diets of this region. The sheep production in Vietnam is variable and has low efficiency due to the dependence on natural feed resources (Khuc Thi Hue, 2012) and there is a lack of staple feeds during the dry seasons. In the Mekong Delta Vietnam, locally natural forages such as Para grass, *Operculumturpethum* and *Psophocarpusscandens* and planted grasses as *Paspalummatratum* and *Pennisetumpurpureum* were widely used for feeding sheep. Silage can be successfully fed to sheep to improve performance, pasture utilization and management by transferring surplus feed to less favorable periods of feed supply (Stanley, 2003). Silages content higher lactic acid compared to grasses which can reduce methane

production in rumen (Cao et al., 2010). Martin et al. (2010) also reported that feeding ruminants with silages tends to reduce methane production compared to this with ground dry roughages or pellets. This study was to evaluate *in vivo* greenhouse gases production as well as identify the feed nutrient utilization and rumen parameters affected by different silages in diets of growing sheep.

MATERIALS AND METHODS

Location and experimental design

The trial was carried out at the experimental farm of CanTho University in CanTho City from Feb 2018 to May 2018. Four Phan Rang male sheep (17,3±1,48kg) were arranged in a Latin Square design with 4 treatments and 4 periods. The four treatments were *Paspalum atratum* (PA) and 40% *Pennisetumpurpureum* silage (PPS40), 40% *Operculinaturpethum* silage (OTS40) and 40% *Psophocarpusscandens* silage (PSS40) replacing *Paspalum atratum* in the diets. Each experimental period was 14 days including 7 days for adaptation and then 7 days for sample collection. The level of different silages replacing *Paspalum atratum* grass in the diets of 40% (DM) was followed the method described by Yulistiani (2016).

Feeds and feeding

The formulation of experimental diets and dietary nutrients and metabolized energy concentration were showed in Table 1.

Table 1. Dietary formula and nutrients and metabolized energy concentration in different diets of the experiment (%DM)

Item	Treatment			
	PA	PPS40	OTS40	PSS40
<i>Paspalum atratum</i> (PA)	81.16	48.94	49.73	50.45
<i>Pennisetumpurpureum</i> silage (PPS)	-	32.62	-	-
<i>Operculinaturpethum</i> silage (OTS)	-	-	33.16	-
<i>Psophocarpusscandens</i> silage (PSS)	-	-	-	33.63
Soybean	17.56	17.19	15.95	14.84
Urea	1.28	1.25	1.16	1.08
CP	19.1	19.19	19.50	19.78
ME, MJ/kg DM	8.59	8.83	9.23	9.15

Note: PA: 100% *Paspalum atratum*, PPS40, OTS40 and PSS40 were *Pennisetumpurpureum* silage, *Operculinaturpethum* silage and *Psophocarpusscandens* silage replacing 40% DM *Paspalum atratum*

The silages were made from *Pennisetumpurpleum* silage, *Operculinaturpethum* silage and *Psophocarpusscandens* collected within the campus of Can Tho University. They were used for feeding sheep from 14 to 21 days after incubation with 11.5% molasses (DM basis) following the suggestions of Nguyen Van Thu (2019). Daily feeds were divided 3 parts and offered the experimental sheep 3 times a day at 7:30, 13:30 and 16:30. *Paspalum atratum* was offered for sheep after they completely consumed the silage. Soybean seeds and urea were bought at feed stores around Can Tho City, in one occasion during experiment. The soybean seeds were roasted and ground before using to feed experimental sheep.

Samplings and Chemical analysis

Feeds, refusal and feces were daily collected, measured and analyzed of DM, OM and Ash following procedure of AOAC (1990), NDF by Van Soest et al. (1991) and metabolizable energy (ME) according to Bruinenberg et al. (2002).

Measurements taken

Daily feed intake and weight gain of sheep in the experiment were measured and calculated every two weeks. Apparent DM, OM, CP and NDF digestibility were done according to McDonald et al. (2002). Rumen fluid was collected via an esophagus tube before and 3 hours post feeding. Total VFAs of rumen fluid was determined following method of Barnet and Reid (1957). Greenhouse gas emissions were measured in 2 consecutive days by chambers, which were designed as method described by Dong Hua Li (2010) and the gas samplings were done by every 30 min. Methane and carbon dioxide concentration of samples were analyzed by the Fast Greenhouse Gas Analyzer, USA.

Statistical analysis

The data were analyzed by analysis of variance using the ANOVA of General Linear Model (GLM) of Minitab Reference Manual Release16.0 (Minitab, 2016). While the Tukey test was used for paired comparison between 2 treatments.

RESULTS AND DISCUSSION

Feed chemical composition and nutrient intake

Chemical compositions of feeds used in the experiment are presented in Table 2.

Table 2. Chemical composition (%DM) of feeds used in the experiment

	DM, %	% DM						pH	VFAs (g/kg)	ME (MJ/kg)
		OM	CP	EE	NDF	ADF	Ash			
PA	18.2	87.0	10.4	4.09	60.8	38.5	13.0	-	-	7.50
PPS	29.9	87.1	10.3	6.47	56.9	37.0	12.9	3.85	27.2	8.32
OTS	27.7	84.5	13.7	7.88	39.8	31.8	15.5	3.80	21.1	9.74
PSS	29.1	86.0	16.2	7.16	43.1	31.6	14.0	4.28	29.4	9.66
Soybean meal	96.9	94.2	41.8	17.2	31.8	24.3	5.85	-	-	13.8
Urea	-	-	288	-	-	-	-	-	-	-

The DM of *Paspalum atratum* (18.2%) was lower than that of the silages (29.9%, 27.7% and 29.1% for PPS, OTS, PSS, respectively). For the forage feeds, PSS and OTS contained the higher CP values compared to PA and PPS, while fiber components (NDF and ADF) was opposite. Soybean meal and urea were used in the experimental diets for supplying crude protein for sheep.

Feed and nutrient intakes

Feeds, nutrients and metabolizable energy intakes of experimental sheep are presented in Table 3.

Table 3. Feeds, nutrients and metabolizable energy intakes of experimental sheep

Item	Treatment				SEM	P
	PA	PPS40	OTS40	PSS40		
<i>Feed intake, gDM/head/day</i>						
PA	467 ^a	312 ^b	344 ^b	324 ^b	16.6	0.002
PPS	-	203	-	-	-	-
OTS	-	-	227	-	-	-
PSS	-	-	-	216	-	-
% silage/PA	-	39.6	40.0	39.9	-	-
<i>Nutrient intake, gDM/head/day</i>						
DM	597	642	690	652	23.6	0.148
OM	529	568	603	574	20.6	0.192
CP	123	127	135	133	4.55	0.327
EE	44.6	49.0	54.2	50.2	2.15	0.095
NDF	321	342	335	323	11.8	0.596
ME, MJ/day	5.47	6.21	6.93	6.32	0.28	0.056
ME, MJ/kgW ^{0.75}	0.574	0.678	0.732	0.678	0.03	0.065

The DM and OM intakes of sheep in the PPS40, OTS40 and PSS40 treatments tended to higher than those of the PA treatment, however, no statistical significance was found ($P > 0.05$). The DM intake of experimental sheep ranged 597-690 g/head/day. This finding was similar to the report of Huynh Hoang Thi (2013), being 476-642 g/head/day by using different levels of *Operculinaturpethum* in growing sheep diets. The OM consumption of sheep in this trial (529-603 g/head/day) consisted with the results of Bekele et al. (2013) being 548-733 g/head/day and Dessie et al. (2009) being 508-680 g/head/day. The CP intakes were 123, 127,

135 and 133 g/day for PA, PPS40, OTS40 and PSS40 treatments, respectively, but no significant ($P>0.05$). The ME consumption of sheep fed the silages (6.21, 6.93 and 6.32 MJ/day corresponding to PPS40, OTS40 and PSS40 treatments) were higher ($P=0.056$) than that of PA treatment (5.47 MJ/day). These values consisted with findings of Paul et al. (2003) stated that growing sheep at 15-20 kg live weight consumed 4.94-9.61 MJ/day. The results in the Table 3 indicated that sheep fed the forage silages in diets at a level of 40% replacement (DM) moderately improved DM, nutrient and ME intakes.

Digestible nutrients, nitrogen balance, daily weight gain and rumen parameters

Table 4. Digestible nutrients, nitrogen balance and daily weight gain of experimental sheep

Item	Treatment				SEM	P
	PA	PPS40	OTS40	PSS40		
<i>Digestible nutrients, g/head/day</i>						
DM	376	433	489	443	22.4	0.060
OM	343	393	440	398	18.7	0.055
CP	103	106	116	114	3.49	0.098
EE	33.0	38.1	44.1	39.7	2.22	0.062
NDF	204	236	234	222	15.1	0.462
<i>Nitrogen balance, g/head/day</i>						
N intake	19.7	20.3	21.6	21.3	0.72	0.298
N feces	3.27	3.37	3.08	3.06	0.34	0.899
N urine	1.50	1.28	1.32	1.33	0.15	0.753
N excrete	4.76	4.65	4.40	4.39	0.32	0.798
N retention	14.9	15.7	17.2	16.9	0.59	0.096
N retention, g/kgW ^{0.75}	1.59 ^b	1.71 ^{ab}	1.83 ^a	1.82 ^a	0.05	0.034
Initial live weight, kg	18.8	18.5	19.1	18.8	0.44	0.831
Final live weight, kg	20.1	19.8	20.7	20.2	0.26	0.223
Daily weight gain, g/day	91.1	94.6	116	96.4	27.7	0.916

The digestible DM, OM, CP, EE and NDF values tended to be higher for the silage treatments compared to the PA one, particularly these were approached a statistical significance ($P\leq 0.06$) for the digestible DM and OM. Thus the use of silages in sheep diets (PPS40, OTS40 and

PSS40 treatments) moderately improved digestible nutrients. The digestible DM and OM values of sheep in this experiment were similar to those reported by Huynh Hoang Thi (2013) with DM and OM digestible being 337-489 and 316-450 g/head/day for the growing sheep, respectively. Nitrogen intake of experimental sheep of PPS40, OTS40 and PSS40 treatments was slightly higher ($P>0.05$) than that of PA treatment (20.3, 21.6 and 21.3 g/head/day vs 19.7 g/head/day). Nitrogen retention (g/head/day) tended to be higher for the silage treatments ($P>0.05$), however nitrogen retention (g/kgW^{0.75}) of OTS40 and PSS40 diets was significantly higher ($P<0.05$) than that of PA treatment. The daily weight gain of sheep in this experiment were not significantly different ($P>0.05$) among treatments although these values were numerically higher for the PPS40, OTS40 and PSS40 treatments.

Table 5. Rumen parameters of pH, N-NH₃ and total VFAs of experimental sheep

Item	Treatment				SEM	P
	PA	PPS40	OTS40	PSS40		
pH at 0 h	6.88	6.80	6.92	6.81	0.06	0.463
pH at 3 h after feeding	6.75	6.77	6.67	6.75	0.04	0.370
N-NH ₃ at 0 h, mg/100ml	24.3	26.6	24.6	26.3	2.56	0.890
N-NH ₃ at 3 h after feeding, mg/100ml	39.9	45.9	42.9	40.8	2.59	0.435
Total VFAs at 0 h, mmol/l	71.8	73.3	69.0	62.8	7.57	0.776
Total VFAs at 3 h after feeding, mmol/l	80.1	82.3	89.1	81.8	6.00	0.737

Note: PA: 100% *Paspalum atratum*, PPS40, OTS40 and PSS40 were *Pennisetum purpureum* silage, *Operculinaturpethum* silage and *Psophocarpus scandens* silage replacing 40% DM *Paspalum atratum*. VFAs: volatile fatty acids

In the Table 5 showed that the pH at 0h and pH at 3h after feeding of rumen fluid of sheep were similar among the treatments ($P>0.05$). The pH at 0h and pH at 3h after feeding ranged 6.8-6.92 and 6.67-6.77, respectively. It indicated that using forage silages for growing sheep did not affect on pH of rumen fluid. The values of pH in present experiment consisted to those reported by Osakwe et al. (2004) and Nguyen Van Thu and Nguyen Thi Kim Dong (2012) being 6.49-6.75 and 6.59-6.80, respectively. The N-NH₃ and VFAs values at 0h and at 3h after feeding of rumen fluid of experimental sheep were not significantly different ($P>0.05$) among treatments. The N-NH₃ and VFAs at 3h after feeding values were higher than compare to these values at 0h. Nguyen Dong Hai (2008) also found that N-NH₃ 0h and at 3h after feeding values of sheep rumen fluid being 24.2-27.7 mg/100ml and 36.4-47.6 mg/100ml, respectively. Similarly, Kusmartono (2007) stated that N-NH₃ at 3h after feeding values of sheep rumen fluid being 37.6-41.3 mg/100ml. The VFAs values at 0h in this experiment were similar to findings of Huynh Hoang Thi (2013) being 62.8-73.3 mmol/l and VFAs at 3h after feeding consisted to those stated by Osakwe et al. (2004) being 80.6-91.6 mmol/l.

Greenhouse gases emissions

The greenhouse gas production by the sheep was presented in Table 6 and Table 7. The methane and carbon dioxide production (l/day) of sheep in this experiment were similar ($P>0.05$) among treatment but these values of PPS40, OTS40 and PSS40 treatments were slightly lower than those of PA treatment. The methane production (g/day) of OTS40 (3.87g/day), PSS40 (4.0 g/day) and PPS40 (4.37 g/day) treatment decreased 21.7%, 19.0% and 11.5% compare to PA treatment (4.94 g/day). The methane production values (g/kg DMI, g/kg OMI) of sheep were significantly different among the treatment ($P<0.05$) with the lowest value for the OTS treatment (Table 6). The methane production (g/kg DMI) of sheep of the PA diets were 20.0%, 28.2% and 34.5% higher compared to PPS40, PSS40 and OTS40 treatments, respectively. Nguyen Van Thu Nguyen Thi Kim Dong (2012) found that using catfish oil at 1-3% levels in growing sheep reduced methane production (g/kg DMI) from 23.3 to 39.5%. The methane production values (g/day, g/kg BW) of experimental sheep were similar to report of Wyatt et al. (2002) being 3.48-5.32 g/day and 0.12-0.60 g/kg BW by using Kikuyu grass for growing sheep.

Table 6. Methane production (g/kgDMI, OMI, DDM, DOM, BW and DWG) of sheep in the experiment

Item	Treatment				SEM	P
	PA	PPS40	OTS40	PSS40		
CH ₄ , L/day	6.89	6.09	5.40	5.57	0.48	0.219
CH ₄ , g/day	4.94	4.37	3.87	4.00	0.34	0.220
CH ₄ , g/kg DMI	8.50 ^a	6.80 ^{ab}	5.57 ^b	6.10 ^{ab}	0.57	0.044
CH ₄ , g/kg OMI	9.60 ^a	7.69 ^{ab}	6.37 ^b	6.93 ^{ab}	0.65	0.050
CH ₄ , g/kg DDM	15.0	10.6	7.87	9.39	1.68	0.097
CH ₄ , g/kg DOM	16.4	11.6	8.74	10.4	1.85	0.104
CH ₄ , g/kg BW	0.250	0.230	0.190	0.200	0.01	0.080
CH ₄ , g/kg DWG	102	51.9	54.5	80.9	41.7	0.813

Note: PA: 100% *Paspalum atratum*, PPS40, OTS40 and PSS40 were *Pennisetum purpureum* silage, *Operculinaturpethum* silage and *Psophocarpus scandens* silage replacing 40% DM *Paspalum atratum*. DMI: Dry matter intake, OMI: Organic matter intake, DDM: Digestible dry matter, DOM: Digestible organic matter, BW: Body weight, DWG: Daily weight gain. The numbers with different superscript letters in the same row were significantly different ($P<0.05$).

In general, CO₂ production values of sheep in present experiment were similar ($P>0.05$) among treatments but those values tended to lower for the PPS40, OTS40 and PSS40 treatments compared to the PA treatment (Table 7). In summary, the results of GHG emissions

indicated that there was a reduction of *in vivo* CH₄ and CO₂ for the silage diets. These findings in the present study were consisted with those found by Cao et al. (2010) and Martin et al. (2010) when feeding silages containing higher lactic acid compared to grasses, which can reduce methane production in rumen.

Table 7. Carbon dioxide production (g/kgDMI, OMI, DDM, DOM, BW and DWG) of sheep in the experiment

Item	Treatment				SEM	P
	PA	PPS40	OTS40	PSS40		
CO ₂ , L/day	121	113	105	113	8.22	0.604
CO ₂ , g/day	239	222	205	222	16.1	0.605
CO ₂ , g/kg DMI	414	347	299	345	28.1	0.127
CO ₂ , g/kg OMI	468	392	341	392	32.0	0.144
CO ₂ , g/kg DDM	742	536	422	528	88.5	0.179
CO ₂ , g/kg DOM	810	587	468	584	96.7	0.189
CO ₂ , g/kg BW	12.3	11.6	10.3	11.3	0.69	0.334
CO ₂ , g/kg DWG	5140	2593	3163	4528	2199	0.833

Note: PA: 100% *Paspalum atratum*, PPS40, OTS40 and PSS40 were *Pennisetum purpureum* silage, *Operculinaturpethum* silage and *Psophocarpus scandens* silage replacing 40% DM *Paspalum atratum*. DMI: Dry matter intake, OMI: Organic matter intake, DDM: Digestible dry matter, DOM: Digestible organic matter, BW: Body weight, DWG: Daily weight gain.

CONCLUSION

It could be concluded that feed and nutrient intakes and digestible nutrients of the growing sheep moderately improved by using silages of *Pennisetum purpureum*, *Operculinaturpethum* and *Psophocarpus scandens* to replace 40% of *Paspalum atratum* (DM basis) in the diets. The use of forage silages at a level of 40% in growing sheep diets reduced methane and carbon dioxide production. Performance studies of feeding silages in growing sheep diets should be implemented to confirm these findings for production recommendations.

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