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ORIGINAL ARTICLE

Role of dose-dependent *Lactobacillus farciminis* on ruminal microflora biogases and fermentation activities of three silage-based rations

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Abstract

Aims: The influence of *Lactobacillus farciminis* on ruminal fermentation characteristics was elucidated in this study.

Methods and Results: Ruminal fermentation was conducted using maize silage ration (R) and concentrate (C) as 75R:25C, 50R:50C and 25R:75C, supplemented with lactic acid bacteria (LB) at 0, 20 and 30 mg g^{-1} dry matter substrate and their interaction (1st experiment). The same LB product was used at 0, 20, 40 and 60 mg g^{-1} dry matter of the mixture (1 : 1) of oat straw and concentrate for 48 h of incubation (2nd experiment).

At 24 and 48 h of incubation, LB0 produced the highest biogas and LB20 produced the lowest, whereas at 48 h of incubation LB40 produced the lowest. In ration x LB, LB40 resulted in the highest biogas production, while LB0 had the lowest ($P < 0{\cdot}001$) at 8, 10 and 12 h of incubation. Inclusions of LB0, 20, 40 and 60 mg g^{-1} dry matter resulted in a linear increase ($P < 0{\cdot}003$) in the asymptotic biogas production and fermentation parameters in a dose-dependent manner, except in pH which decreased ($P = 0{\cdot}029$).

Conclusions: The use of *L. farciminis* in diet with high level of concentrate without any adverse effect on the pH of rumen fluid to the point of acidosis. Furthermore, in high forage diet, the use of *L. farciminis* would help to improve the ruminal fermentation digestibility and mitigate ruminal biogas production.

Significance and Impact of the Study: Using *Lactobacillus* as a feed additive can improve ruminal fermentation activities by maintaining the stability of pH in the rumen and improving the feed utilization through manipulation of the microbial ecosystem.

Introduction

The importance of ruminants to human nutrition, income, employment and raw materials for agro-based industry in the form of meat, milk, leather, bones cannot be underscored. The quality and quantity of these benefits are influenced by how efficient ruminants can digest their diet. *Lactobacillus* have proved to be capable of improving the rumen environment through rumen pH stability, feed utilization through manipulation of microbial ecosystem (Astuti *et al.* 2018) and improve beneficial microbes in the rumen.

Feed digestibility and rumen fermentation characteristics can be improved by manipulation of the rumen environment. Poor digestibility is one of the main challenges in ruminant nutrition especially when they are fed fibre-containing diet. The continual interest among animal nutritionists is to improve the feed efficiency and animal performance. The motive of improving digestibility, productivity and feed efficiency through rumen manipulation; has led to the continual use of antibiotics which resulted in the development of resistance among microbes (Adegbeye *et al.* 2018). This consequently led to the regulation, to ban/control the use of ionophores and medically important antibiotics. To improve the productivity while maintaining 'clean' animal productivity, there is need for a suitable alternative.

There is a growing research interest in the application of beneficial microbes/probiotics in ruminant production (Adjei-Fremah *et al.* 2018) for improving gut health, productivity, rumen manipulation, and perhaps, for reducing greenhouse gas emission. Probiotics are live organisms that are given to animals to confer beneficial effect on the host. Hence, the use of probiotics in improving the digestibility and performance of livestock may be a suitable alternative to the use ionophores and other chemical additives. Most of the probiotic bacterial genera are not 'foreign' to the gut environment. *Lactobacillus* sp., *Weissella*, *Aerococcus*, *Bifidobacterium* and *Enterococcus* have been used as probiotics with beneficial effects on the host (Uyeno *et al.* 2015). The effects could be in the form of microbial stability, improving digestibility, reducing or preventing establishment of pathogens, preventing acidosis and enhancing the growth of beneficial microflora population (Izuddin *et al.* 2018). Lactic acid bacteria (LB) ferment carbohydrate to produce lactic acid (Astuti *et al.* 2018). The supplementation with *Lactobacillus* could improve rumen fermentation activities, and interestingly, reduce methane emission (Astuti *et al.* 2018; Wingard *et al.* 2018).

In addition, *Lactobacillus plantarum* strain (Astuti *et al.* 2018) has been shown to reduce the negative environmental impact such as methane emission (Adjei-Fremah *et al.* 2018). This is because acetic acid, formic acid, hydrogen peroxide and β-hydroxy-propionaldehyde (reuterin) produced alongside lactic acid by *Lactobacillus* act as antibacterial agent (Takahashi 2013). Hence, the direct involvement of low-molecular hydrogen peroxide may be the mechanism for its rumen methane inhibition. In addition, a protease-resistant antimicrobial compound (PRA-1) is produced by *Lactobacillus*. *Lactobacillus plantarum* TUA1490L is capable of inhibiting or reducing methane production (Takahashi *et al.* 2005; Asa *et al.* 2010). However, there is little or no information on the use of *Lactobacillus farciminis* on ruminant fermentation activity, especially their impacts on production of biogases. Hence, the aim of this study was evaluate the role of *L. farciminis* on the rumen biogas and fermentation characteristics of rations with varying levels of silage to concentrate.

Materials and methods

Preparation of lactic acid bacterial culture broth

Lactobacillus farciminis (3×10^{11} CFU per gram; a commercial product of SAFISIS, Toluca, Mexico) was activated in a rumen medium of Goering and Van Soest (1970) buffer solution a day prior to experiments. Lactic acid bacteria were added to 1% (v/v) rumen medium in a 1-l flask, well mixed and incubated under static conditions at 39°C for 24 h in a water bath, after saturation with CO_2 for 10 min.

Substrate and treatments

A mixture of three total mixed ration (TMR) of maize silage (R): concentrate (C) were prepared in a ratio (25R:75C, 50R:50C and 75R:25C) with three doses of lactic acid bacteria (0 (LB0), 20 (LB20) and 40 (LB40) mg g^{-1} dry matter (DM) of TMR as a substrate) used in the first *in vitro* ruminal fermentation experiment (Table 1 and Table 2). In the second *in vitro* experiment, four doses of 0 (LB0), 20 (LB20), 40 (LB40) and 60 (LB60) mg g^{-1} DM of the oat straw and concentrate (1 : 1) as a substrate) were used during the ruminal biogas incubation (Table 3).

Biogas production

Rumen fluid was collected from two ruminally cannulated Holstein steers (450 ± 20 kg body weight) fed a TMR, formulated based on the NRC (2001) requirements *ad libitum*, made of alfalfa concentrate and commercial concentrate (PURINA®, Toluca, Mexico) in a 1 : 1 ratio. The rumen contents were collected before feeding and

Table 1 Chemical composition* (g kg^{-1} DM) of three rations with different silage (R) and concentrate† (C) ratios and total mixed ration for the second experiment (g kg^{-1} DM)

Ration	Organic matter	Crude protein	Neutral detergent fibre	Acid detergent fibre
25F:75C	932·6	133·2	217·7	88·2
50F:50C	939·6	138·7	302·2	127·0
75F:25C	943·7	92·0	371·7	149·0

*Contained (g kg^{-1}): 200 maize grain flacked, 260 maize grain cracked, 154 sorghum grain, 100 molasses sugarcane, 100 distilled dry grain, 96 soya bean meal, 70 wheat bran, 10 $NaCOOH_3$, 10 mineral mixture: *Mineral/vitamin premix*: vitamin A (12 000 000 IU), vitamin D3 (2 500 000 IU), vitamin E (15 000 IU), vitamin K (2·0 g), vitamin B1 (2·25 g), vitamin B2 (7·5 g), vitamin B6 (3·5 g), vitamin B12 (20 mg), pantotenic acid (12·5 g), folic acid (1·5 g), biotin (125 mg), niacin (45 g), Fe (50 g), Zn (50 g), Mn (110 g), Cu (12 g), I (0·30 g), Se (200 mg), Co (0·20 g).

†Mixture of 50% commercial concentrate with 50% wheat bran.

Table 2 Rumen biogas kinetics and total cumulative biogas production after 48 h of incubation of three different mixture ratios of maize silage (R) with concentrate (C) as affected by different levels of lactic acid bacteria (LB, mg g^{-1} DM of substrate)

		Biogas kinetics			Biogas production (ml g^{-1} DM) at (h):								Fermentation profile							
Ration	LB	*A*	*c*	*L*	2	4	6	8	10	12	24	48	pH	ME	DMD	OMD	SCFA	PF_{24}	MCP	GY_{24}
75R:25C	0	322·4	0·178	1·45	85·3	143·8	184·6	213·9	235·4	251·5	297·3	318·4	6·62	11·0	786·1	741·7	6·58	5·02	832·0	199·3
	20	219·5	0·318	1·27	103·3	158·0	186·9	202·2	210·3	214·6	219·4	219·5	6·61	8·9	790·8	603·1	4·85	5·33	686·2	187·7
	40	283·2	0·297	1·47	126·9	196·8	235·4	256·8	268·6	275·1	282·9	283·2	6·65	10·7	803·3	716·2	6·26	5·05	805·1	198·2
	Linear	0·441	0·035	0·959	0·009	0·008	0·005	0·002	0·001	0·004	0·627	0·457	0·470	0·627	0·229	0·627	0·627	0·770	0·627	0·744
	Quadratic	0·090	0·078	0·480	0·779	0·334	0·066	0·004	<0·001	<0·001	0·027	0·078	0·566	0·027	0·739	0·027	0·027	0·006	0·027	0·008
50R:50C	0	319·5	0·166	1·51	81·5	139·2	180·5	210·7	233·0	249·9	296·8	316·2	6·61	11·1	774·5	742·8	6·57	5·02	830·9	199·4
	20	263·2	0·223	1·79	94·5	155·0	193·8	218·6	234·5	244·7	261·8	263·2	6·65	10·1	751·2	680·7	5·79	5·12	765·6	195·2
	40	274·5	0·244	1·41	105·8	170·8	210·8	235·3	250·4	259·7	273·7	274·5	6·62	10·4	730·2	701·7	6·05	5·08	787·8	196·9
	Linear	0·326	0·080	0·584	0·033	0·030	0·022	0·010	0·001	0·034	0·391	0·330	0·769	0·391	0·064	0·390	0·391	0·437	0·391	0·428
	Quadratic	0·389	0·595	0·063	0·913	0·998	0·831	0·468	0·031	0·018	0·321	0·382	0·328	0·321	0·948	0·321	0·321	0·270	0·321	0·282
25R:75C	0	389·4	0·076	1·50	54·8	101·9	142·3	177·1	206·9	232·6	326·1	379·0	6·60	11·6	712·0	773·7	7·22	4·92	885·8	203·4
	20	367·8	0·129	1·59	75·9	132·9	176·5	210·5	237·4	259·1	328·6	362·2	6·67	11·7	685·5	778·2	7·27	4·91	890·6	203·5
	40	268·8	0·271	1·76	112·6	177·9	216·0	238·1	250·9	258·4	268·4	268·8	6·71	10·0	690·9	671·1	5·94	5·10	778·0	196·1
	Linear	0·008	0·003	0·386	0·004	0·005	0·006	0·007	0·010	0·018	0·009	0·008	0·116	0·009	0·295	0·009	0·009	0·005	0·009	0·005
	Quadratic	0·197	0·240	0·873	0·515	0·662	0·868	0·834	0·437	0·098	0·054	0·170	0·736	0·054	0·354	0·054	0·054	0·041	0·054	0·043
Pooled SEM		28·82	0·0285	0·179	7·81	10·19	9·62	7·62	5·34	4·18	16·53	26·82	0·036	0·45	12·16	29·39	0·367	0·048	30·91	1·93
P value																				
Ration:																				
Linear		0·011	<0·001	0·145	0·001	0·003	0·007	0·021	0·164	0·396	0·007	0·010	0·303	0·027	<0·001	0·037	0·007	0·001	0·007	0·001
Quadratic		0·278	0·957	0·604	0·880	0·668	0·495	0·356	0·260	0·342	0·417	0·292	0·548	0·721	0·413	0·790	0·417	0·573	0·417	0·526
LB:																				
Linear		0·010	<0·001	0·693	<0·001	<0·001	<0·001	<0·001	<0·001	<0·001	0·030	0·011	0·086	0·030	0·123	0·030	0·030	0·035	0·030	0·034
Quadratic		0·216	0·386	0·801	0·566	0·382	0·191	0·046	0·002	<0·001	0·090	0·203	0·667	0·090	0·426	0·090	0·090	0·013	0·090	0·021
Ration × LB		0·119	0·064	0·427	0·326	0·305	0·174	0·028	<0·001	<0·001	0·024	0·101	0·555	0·024	0·178	0·024	0·024	0·002	0·024	0·004

A = asymptotic biogas production (ml g^{-1} DM); *c* = rate of biogas production (/h); *L* = the initial delay before biogas production begins (h).

DMD = dry matter disappearance (mg g^{-1} DM); GY_{24} = biogas yield at 24 h of incubation (gas per gram DMD); MCP = microbial crude protein biomass production (mg g^{-1} DM); ME = metabolizable energy (MJ kg^{-1} DM); OMD = organic matter digestibility (mg g^{-1} DM); PF_{24} = partitioning factor at 24 h of incubation (mg DMD per ml gas); SCFA = short-chain fatty acids (mmol g^{-1} DM).

Table 3 Rumen biogas kinetics and total cumulative biogas production after 48 h of incubation of a total mixed ration of oat straw and concentrates (1 : 1) as affected by different levels of lactic acid bacteria (LB, mg g^{-1} DM of substrate)

	Biogas kinetics			Biogas production (ml g^{-1} DM) at (h):								Fermentation profile							
LB	*A*	c	*L*	2	4	6	8	10	12	24	48 h	pH	ME	DMD	OMD	SCFA	PF_{24}	MCP	GY_{24}
0	150·6	0·136	1·56	35·6	62·6	83·2	98·8	110·8	120·0	144·1	150·3	6·82	7·01	499·5	480·1	3·18	5·99	545·4	167·0
20	166·2	0·132	1·79	38·5	68·0	90·7	108·2	121·6	131·9	159·1	165·9	6·80	7·42	468·9	506·8	3·51	5·81	573·5	172·2
40	192·0	0·141	1·76	47·0	82·5	109·3	129·5	144·8	156·3	185·3	191·8	6·73	8·14	476·5	553·5	4·09	5·56	622·6	179·8
60	208·8	0·148	1·91	53·6	93·4	123·0	145·0	161·4	173·5	202·8	208·6	6·73	8·61	503·5	584·5	4·48	5·43	655·2	184·1
SEM	5·90	0·0079	0·082	1·54	2·46	3·02	3·41	3·72	4·00	5·23	5·85	0·026	0·142	12·45	9·31	0·116	0·054	9·78	1·60
Linear	0·001	0·709	0·128	0·001	<0·001	<0·001	<0·001	<0·001	<0·001	0·001	0·001	0·029	0·001	0·228	0·001	0·001	0·001	0·001	0·001
Quadratic	0·498	0·501	0·251	0·175	0·172	0·177	0·190	0·211	0·238	0·406	0·492	0·451	0·405	0·245	0·408	0·405	0·628	0·406	0·552

A = asymptotic biogas production (ml g^{-1} DM); *c* = rate of biogas production (/h); *L* = the initial delay before biogas production begins (h).
DMD = dry matter disappearance (mg g^{-1} DM); GY_{24} = biogas yield at 24 h of incubation (biogas per gram DMD); MCP = microbial crude protein biomass production (mg g^{-1} DM); ME = metabolizable energy (MJ kg^{-1} DM); OMD = organic matter digestibility (mg g^{-1} DM); PF_{24} = partitioning factor at 24 h of incubation (mg DMD per ml gas); SCFA = short-chain fatty acids (mmol g^{-1} DM).

strained through four layers of cheesecloth into a flask with O_2-free headspace. One gram of the TMR was weighed into 120-ml serum bottles after adding LB doses per gram DM. Subsequently, 10 ml of rumen fluid and 40 ml of the buffer solution was added to each serum bottle (Goering and Van Soest 1970), with exception to trypticase. The bottles were closed with a rubber stopper, shaken and incubated at 39°C, and the biogas volumes were recorded at 2, 4, 6, 8, 10, 12, 14, 24 and 48 h of incubation. The pressure reading technique (Extech Instruments, Waltham, MA) of Theodorou *et al.* (1994) was used for biogas production recordings. After 48 h of incubation, the pH was measured using a pH meter (Conductronic pH15, Puebla, Mexico) after the bottles were uncapped while the undigested residue was obtained after the content of the bottles were filtered. The analysis and degradability of samples were done as described by Elghandour *et al.* (2014).

Chemical analyses and calculations

The DM (#934·01), ash (#942·05) and N (#954·01) of TMR were analysed using the AOAC (1997) method. Neutral detergent fibre (NDF) and acid detergent fibre (ADF—lignin) were analysed using the method of Van Soest *et al.* (1991) and AOAC (1997; #973·18) with an ANKOM200 Fiber Analyzer Unit (ANKOM Technology Corp., Macedon, NY). Neutral detergent fibre was assayed using alpha-amylase and sodium sulphite. Both NDF and ADF are expressed without residual ash (Table 1).

The results of kinetic parameters of gas production (GP; ml g^{-1} DM) were fitted using the NLIN option of Co SAS (2002) according to France *et al.* (2000) as follows:

$$A = b \times \left(1 - e^{-c(t-L)}\right)$$

where *A* is the volume of GP at time t, *b* is the asymptotic GP (ml g^{-1} DM), *c* is the rate of GP (ml h^{-1}) and *L* (h) is the discrete lag time prior to initiation of biogas production.

The estimation of *in vitro* organic matter digestibility (OMD, g kg^{-1} OM) and metabolizable energy (ME, MJ kg^{-1} DM) were done according to Menke *et al.* (1979) as follows:

$$\begin{aligned} \mathrm{ME} &= 2 \cdot 20 + 0 \cdot 136\ \mathrm{GP}\ (\mathrm{ml}/0 \cdot 5\ \mathrm{g\ DM}) \\ &\quad + 0 \cdot 057\ \mathrm{CP}\ \left(\mathrm{g\ kg^{-1}\ DM}\right) \\ \mathrm{OMD} &= 148 \cdot 8 + 8 \cdot 89\ \mathrm{GP} + 4 \cdot 5\ \mathrm{CP}\ \left(\mathrm{g\ kg^{-1}\ DM}\right) \\ &\quad + 0 \cdot 651\ \mathrm{ash}\ \left(\mathrm{g\ kg^{-1}\ DM}\right) \end{aligned}$$

where GP is net GP in ml from 200 mg of dry sample after 24 h of incubation.

The ratio of *in vitro* DM degradability (DMD, mg) to the volume (ml) of GP at 24 h (i.e. DMD/total biogas production (GP_{24}) were used to estimate the partitioning factor at 24 h of incubation (PF_{24}; a measure of fermentation efficiency) according to Blümmel *et al.* (1997). Biogas yield (GP_{24}) was calculated as the volume of biogas (ml gas g^{-1} DM) produced after 24 h of incubation divided by the amount of DMD (g) as follows:

$$\text{Biogas yield } (GY_{24}) = \text{ml biogas/g DM/g DMD}$$

Short-chain fatty acid (SCFA) concentrations were calculated according to Getachew *et al.* (2002) as follows:

$$\text{SCFA (mmol/200 mg DM)} = 0 \cdot 0222\ \text{GP} - 0 \cdot 00425$$

where GP is the 24-h net biogas production (ml per 200 mg DM).

Microbial crude protein biomass production (MCP) was calculated according to Blümmel *et al.* (1997) as follows:

$$\text{MCP}\left(\text{mg}\,\text{g}^{-1}\,\text{DM}\right) = \text{milligrams DMD} - \left(\text{millilitre biogas} \times 2 \cdot 2\,\text{mg}\,\text{ml}^{-1}\right).$$

where the 2·2 mg ml^{-1} is a stoichiometric factor that expresses milligrams of C, H and O required for the SCFA biogas associated with production of 1 ml of biogas (Blümmel *et al.* 1997).

Statistical analyses

The average of the data for each of the three runs within the same sample of each of the three individual samples of TMR was used for statistical analysis. Mean values of each individual sample were used as the experimental unit. The biogas production and rumen fermentation parameters results were analysed as a factorial experiment using the PROC GLM option of Co SAS (2002) as:

$$Y_{ije} = \mu + D_i + B_j + \varepsilon_{ije}$$

where Y_{ije} is every observation of the i_{th} diet (D_i) with j_{th} LB dose (B_j), μ is the general mean, D_i (i = 1–3) is the TMR of different maize silage concentrate ratios, B_j (j = 1–4) is the algae doses effect and $\mathcal{E}_{ije}$ is the experimental error.

Results

Effect of ration

The ration had a linear effect ($P = 0{\cdot}011$) on the asymptotic biogas production and rate of biogas production. Biogas production increased ($P < 0{\cdot}020$) with decreasing fibre content in the diet while the rate of biogas production decreased linearly ($P < 0{\cdot}001$) with increasing level of concentrate. There was a linear ($P < 0{\cdot}03$) increase in *in vitro* biogas production at 2, 4 6, 8, 10, 12, 24 and 48 h of incubation with increasing concentrate proportion per substrate.

Dry matter degradability decreased linearly ($P < 0{\cdot}001$) with increasing level of concentrate. In contrast, OMD, SCFA and MCP values increased linearly ($P < 0{\cdot}04$) with increasing level of concentrate in the substrate during digestion. Partitioning factor at 24 h of incubation decreased linearly ($P = 0{\cdot}001$) as the concentrate increased and gas yield in 24 h increased linearly ($P = 0{\cdot}001$) as the roughage percentage decreased in the diet (Table 2).

Effect of lactic acid bacteria

Asymptomatic GP decreased linearly ($P < 0{\cdot}001$) with increasing LB in a dose-dependent manner. However, the rate of biogas production increased linearly ($P < 0{\cdot}001$) with increasing concentrate and LB40 had the highest rate of biogas production per hour.

The inclusion of LB had a linear and quadratic effect ($P < 0{\cdot}05$) on the *in vitro* GP production. The inclusion of LB40 produced the highest biogas in 2–12 h of incubation. However, at 24 and 48 h of incubation, the inclusion of LB0 produced the highest biogas. In addition, the inclusion of LB0, LB40 and LB20 had a linear ($P = 0{\cdot}030$) effect on OMD and SCFA and decreased accordingly (Table 2).

Ration and lactic acid bacteria interaction

In all rations, LB40 produced the highest ($P < 0{\cdot}001$) biogas production at 8, 10 and 12 h of incubation. At 24 h, LB0 had the highest ($P = 0{\cdot}024$) biogas production for all rations except in R25:75C where LB20 was the highest. Furthermore, LB0 had the highest while LB20 had the lowest ($P = 0{\cdot}024$) OMD, ME, SCFA and MCP in 75R:25C and 50R:50C. In contrast, LB20 had the highest ($P = 0{\cdot}024$) in 25R:75C for OMD, ME, SCFA and MCP (Table 2).

Ruminal biogas kinetics and production

Lactic acid bacteria had a linear effect on the biogas kinetics and fermentation profile of oat straw and concentrate. The result showed that there was dose-dependent increase in asymptomatic biogas production (ml g^{-1} DM). Similarly, ME, OMD, SCFA and MCP increased linearly ($P = 0{\cdot}001$) in a dose-dependent manner. In contrast, pH decreased linearly ($P = 0{\cdot}029$) with increasing level of LB except in LB40 and LB60 which had the same values. However, LB had no effect on DMD of substrate, rate of biogas production (/h) and lag time (Table 3).

Discussion

Nutrient recycling such as the use of agricultural by-product could reduce environmental pollution and perhaps reduce the pressure on human edible ingredient fed to animal. The feeding of agricultural by-product such as crop residue, and fruit and vegetable waste is practised in many developing countries. Ruminant are excellent options/livestock that can be help to convert low-quality protein diet into high-quality protein diet. However, poor digestibility causes inefficiency in deriving nutrient from such ingredient due to their lignicellulolisic nature. Pretreatment with chemical or fungi is also an alternative good option. However, pre-acidic or alkaline treatment is costly, environmentally unfriendly and unsuitable for the ensiling process (Keller *et al.* 2003). Alternatively, the use of LB can make the biosilage process simpler, faster, more environmentally friendly and cost-efficient than chemical technology (Novik *et al.* 2017). Before, recommending LB for use, *in vitro* digestion is needed. The biogas production will be to measure the degree of digestibility and the ability of the microbes to quickly adapt, adapt to the substrate and colonize it to break it down.

Effect of ration

Silages consist of high fibrous content than concentrate, which consist of rapidly digestible constituent. Thus, the increase in biogas production may be attributed to the quick digestibility, which might have occurred because microbes in liquors were able to breakdown the most substrate available rather than spend longer period breaking down the complex polymer of the cell wall in roughages. The rate of digestibility also reflects in the rate at which gas was produced per hour and the higher OMD with increasing concentrate. The chemical composition of the high concentrate diet indicates the NDF and ADF was low while the crude protein (CP) would favour the proliferation of rumen fluid microbes due to availability of ammonia nitrogen. The rapid breakdown of higher concentrate diet also reflects the availability of ME. The increase in microbial crude protein biomass production (MCP) with increasing concentrate may be attributed to the CP content in the diet, which provided nitrogen for the proliferation of rumen microbes that serves as a source of microbial protein.

Short-chain fatty acids are by-products of microbial fermentation of organic matter, which usually occur, in anaerobic condition. Short-chain fatty acids provide energy needed by ruminant for production. The increase in SCFA with decreasing roughage indicates that there was higher digestibility, which enhanced microbial proliferation and metabolites. Ruminal pH is a parameter that indicates the state of acidity and alkalinity of the rumen, and could be used to predict the type of diet fed to animal (Faniyi *et al.* 2019). In this study, despite the increase in digestibility of high- concentrate diet, the pH was within the optimal range of 6·0–6·8 (Kamra 2005; Ososanya *et al.* 2013). The possible reason for the optimal range of pH in the rumen even in high-concentrate diet is that, the diet might have favoured protozoa popoulation (Leng 2014), which could have swallowed soluble starch granules (Rode 2000). Hence, the pH in the rumen is regulated.

Effect of lactic acid bacteria

The LB0 produced the highest asymptomatic biogas and the rate of biogas production occurred at the shortest time compared to other treatments with LB inclusion. The possible reason for this is the inclusion of *Lactobacillus* higher than the optimal level required for the activation of rumen microbes, thus acting as an antibacterial agent against rumen microbes instead of improving the beneficial microbes. *Lactobacillus* can secrete bacteriocins and hydrogen peroxide, which are antimicrobial peptides (Choe *et al.* 2013; O'Brien *et al.* 2013).

The increase in GP with LB40 during the first 12 h of incubation may be attributed to the participation of LB in aiding the quick degradation of soluble nutrient available in a short period of time. However, the increase in *in vitro* GP in LB0 during 24 and 48 h may be attributed to the antimicrobial activity of *Lactobacillus* on the rumen microbes such as the secondary colonizers which are more proficient at digesting starch and cell walls of plants (Huws *et al.* 2016) or the exhaustion of the soluble substrate by the rumen microbes within a short period, while the slow rate of GP in the LB0 enabled them to have more substrate available for degradation over a longer period of time. The *Lactobacillus* might have acted as a probiotic or catalyst to the microbes during the early state of fermentation, which reflects in the rate of GP per hour. However, LB0 had higher ME, SCFA and MCP values than other LB inclusions. This might be due to the availability of more substrate for digestion over a prolonged period, as reflected on the low rate of GP per hour.

Ration and lactic acid bacteria interaction

The influence of lactate-producing bacteria on biogas production is dependent, at least in part, on time of incubation and substrate fermented (Wingard *et al.* 2018). The higher biogas production in LB40 may be attributed to the higher number of LB number in the rumen liquor which resulted in increased fermentation (Russell and Wilson 1996). The reason for decrease in GP in LB supplementation after 12 h may be attributed to reduction in soluble carbohydrate after the initial quick degradation, which resulted in the slowdown of biogas production. However, LB inclusion did not outperform the LB0 with regard to ME, OMD, SCFA, MCP and biogas yield.

Ruminal gas kinetics and total cumulative production

Probiotics for adult ruminants have mainly been selected to improve fibre digestion by rumen micro-organisms (Uyeno *et al.* 2015). Furthermore, it has been suggested that the influence of *Lactobacillus* during digestion is dependent on dosage or level (Jiao *et al.* 2017; Izuddin *et al.* 2018).

There was a general increase in asymptomatic biogas, ME, OMD, SCFA and MCP with increasing dosage of LAB. This confirms the ability of LAB to improve or stimulate the growth of rumen microbes to improve digestibility and nutritional benefit derivable from it. In addition, the fibre digestion might also be due to the production of feruloyl esterases by *L. farciminis.* Xu *et al.* (2017) reported that breaking of ferulic acid linkages could help make cell wall susceptible to ruminal digestion. Although the pH was not in any way close to acidosis, the decrease in pH may be attributed to the presence of glucogenic volatile fatty acid such as lactic acid, propionic acid due to the increasing presence of LB. Despite the increasing digestion due to dosage of LB, the decrease in pH may be attributed to the increased stimulation of lactic acid utilizing bacteria such as *Megasphaera*. This confirms that it is possible for lactic acid to digest starch without causing acidosis (Yang *et al.* 2018). Thus, *L. farciminis* may be included in ruminant diet containing high starch/concentrate without having negative effect on the pH of the rumen fluid.

The inclusion of higher level (LB40 and LB60) could be used in diet containing high forages in order to OMD (and indicator of digestibility) SCFA, ME availability and aid the proliferation of microbes which favours the synthesis of microbial protein. In addition, the antimicrobial properties in these lactic acid bacteria might make it a useful tool as probiotics against protozoa and methanogens in ruminant. In conclusion, *L. farciminis* has the ability to improve the rumen environment, feed digestibility and SCFA production without disrupting the rumen pH during fermentation. Similarly, LB could improve rumen microbial population, which will aid efficient nutrient use by the ruminant.

Conflict of Interest

The authors declare that they have no conflict of interest.

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