Journal of Applied Animal Research

ISSN: 0971-2119 (Print) 0974-1844 (Online) Journal homepage: http://www.tandfonline.com/loi/taar20

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To cite this article: M. M. Y. Elghandour, J. C. Vázquez, A. Z. M. Salem, A. E. Kholif, M. M. Cipriano, L. M. Camacho & O. Márquez (2017) In vitro gas and methane production of two mixed rations influenced by three different cultures of Saccharomyces cerevisiae, Journal of Applied Animal Research, 45:1, 389-395, DOI: 10.1080/09712119.2016.1204304

To link to this article: http://dx.doi.org/10.1080/09712119.2016.1204304

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Published online: 05 Jul 2016.

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JOURNAL OF APPLIED ANIMAL RESEARCH, 2016
VOL. 45, NO. 1, 389–395
http://dx.doi.org/10.1080/09712119.2016.1204304

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In vitro gas and methane production of two mixed rations influenced by three different cultures of *Saccharomyces cerevisiae*

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ABSTRACT
The current study aimed to evaluate if the effect of *Saccharomyces cerevisiae* (SC) on *in vitro* fermentation can be affected with the crude protein (CP) content of the ration. Three commercial SC cultures of Biocell F53®, Procreatin 7®, and Biosaf SC47® were evaluated at 0 (SC0), 2 (SC2), and 4 (SC4) mg/g dry matter (DM) of substrate. Two rations with 13% (low crude protein [LCP]) and 16% CP (high crude protein [HCP]) were used as substrates. Rumen gas (gas production [GP]) and methane (CH_4) productions were recorded. The HCP ration had increased ($P = .05$) asymptotic GP, CH_4 production, and fermentation parameters. Biocell F53® and Biosaf SC47® increased the asymptotic GP ($P < .05$) in HCP and LCP rations with better effect for the dose of 2 mg/g DM substrate HCP ($P < .05$) and dose of 4 mg yeast/g DM substrate with the LCP ration. The highest CH_4 production was observed ($P < .05$) with Procreatin 7®. It could be concluded that HCP ration improved GP than LCP ration. Moreover, addition of Biocell F53® and Biosaf SC47® at rate of 2 mg/g DM improved fermentation kinetics and nutrients degradability.

Abbreviations: *b*: the asymptotic gas production; *c*: the rate of gas production; CH_4: methane; GY_{24}: gas yield at 24 h of incubation; *L*: the initial delay before gas production begins; MCP: microbial CP production; PF_{24}: partitioning factor at 24 h of incubation.

ARTICLE HISTORY
Received 10 January 2015
Accepted 9 June 2016

KEYWORDS
Degradability; methane; protein level; yeast

1. Introduction

One of the most important problems facing ruminant production is the losing of energy and high biological value proteins as a result of ruminal fermentation. This may cause a limited productive performance (Kholif et al. 2014; Ahmed et al. 2016) and release of pollutants to the environment (Calsamiglia et al. 2007). Ionophores and antibiotics have good results to reduce these losses in energy and protein (McGuffey et al. 2001); however, the European Union banned the use of them due to the potential of appearance of residues in milk or meat (Russell and Houlihan 2003). Nowadays, researches are concerning the use of natural feed additives, generally recognized as safe for human consumption, including phytogenic extracts (Valdes et al. 2015), enzymes (Alsersy et al. 2015) or *Saccharomyces cerevisiae* (SC) (Elghandour, Salem et al. 2015) to modify rumen microbial fermentation.

The SC is generally recognized as safe by the US Food and Drug Administration, and they can be legally used as animal feed additives. Yeast, as a natural feed additive, has the ability to stabilize rumen fermentation and prevents rumen flora disorders and disturbances (Pinloche et al. 2013) with increasing the numbers of viable bacterial cells (Jouany 2001). Enhanced ammonia utilization by ruminal microorganisms is another benefit from using yeast (Chaucheyras-Durand et al. 2008). Moreover, SC can provide the rumen with important nutrients and nutritional cofactors in addition to vitamins, which reported to be required for microbial growth and activity (Mao et al. 2013; Polyorach et al. 2014). The SC have the ability to increase dry matter (DM) and neutral detergent fibre (NDF) digestion (Elghandour et al. 2014, Elghandour, Salem et al. 2015), and increase initial rates of fibre digestion (Williams et al. 1991). In addition, it could enhance fungal colonization of plant cell walls, resulting in increased DM and NDF digestion (NDFD) (Patra 2012), and improved *in situ* crude protein (CP) and NDF degradation. Elghandour et al. (2014) reported an increased *in vitro* rumen degradability of forages, which was associated with ability of yeast to stimulate growth and activity of fibrolytic bacteria (Wambui et al. 2010).

Increased gas production (GP) was paralleled with administration of SC (Elghandour et al. 2014), which might stimulated the acetogens to compete or co-metabolize hydrogen with methanogens, thereby reduce methane (CH_4) emissions (Hristov et al. 2013). However, others reported increased CH_4 emission (Martin and Nisbet 1992), or not affected (Mathieu et al. 1996) with SC administration. These conflicting results on CH_4 emission are likely due to strain difference of SC and type of diets (Patra 2012). In general, there is inconsistence between reports regarding the effect of yeast on animals' performance. Some of the possible causes for the inconsistency could be associated with characteristics of the strain

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(Newbold et al. 1996), differences between commercial additives (Mendoza et al. 1995), and diet composition (Elghandour et al. 2014).

The commercially available SC cultures, in general, contain mixtures of varying proportions of live and dead cells. So, as expected, the response to different SC cultures will vary depending on number of live or metabolically active SC cells, the dose used, the feeds, and/or other nutrients compounds in the cultures such as fats, proteins, ash, and carbohydrates (Elghandour et al. 2014). Therefore, the current study aimed to study the effect of three SC cultures, abundant in Mexico, at different doses on GP and fermentation kinetics of two total mixed rations with high (16% CP; high crude protein [HCP]) and low (13% CP; low crude protein [LCP]) CP levels.

2. Materials and methods

2.1. Substrates and *yeast levels*

Two mixed rations with two different levels of CP of 13% (LCP) and 16% (HCP) on DM basis (Table 1) were used as substrates to be incubated with three doses of different SC *culture*s.

Three types of SC cultures were tested at three doses (mg/g DM of substrate): 0 (without; SC0), 2 (SC2), and 4 (SC4). Stock solution of each yeast culture doses was prepared before treatments in distilled water in order to get the suitable doses in 1 ml of each stock solution.

The three cultures of SC (Lesaffre Feed Additives, Toluca, Mexico) were used: (1) Biocell® contains a minimum guarantee of 2.0×10^{10} CFU/g *SC*. (2) Procreatin 7® contains minimum guarantee of 1.5×10^{10} CFU/g *SC*. (3) Biosaf SC47® contains, as a minimum count of live yeast cell 1.0×10^{10} CFU/g *SC*.

2.2. In vitro *incubations*

As described before in Elghandour et al. (2014), three sheep (35 to 45 kg body weight) fitted with permanent rumen cannula were used as rumen inoculum donors and fed on a total mixed ration of commercial concentrate and corn silage at 1:1 DM formulated to cover their nutrient requirements (NRC 1985). Sheep had a free access to fresh water during all times of rumen inoculum collection phase.

Before the morning feeding, ruminal contents were obtained from each sheep and flushed with CO_2 to keep it anaerobically, then mixed and strained through four layers of cheesecloth into a flask with O_2 free headspace. Feeds samples (0.5 g) were weighed into 120 ml serum bottles with appropriate addition of yeast cultures doses/g DM. About 10 ml of particle free ruminal fluid were added to each bottle followed by 40 ml of the buffer solution according to Goering and Van Soest (1970), with no trypticase added, in a 1:4 (vol/vol) proportion.

Once all bottles were filled, they were immediately closed with rubber stoppers, shaken and placed in the incubator at 39°C. The volume of gas produced and CH_4 production were recorded at times of 2, 4, 6, 8, 10, 12, 14, 24, and 48 h of incubation. GP was recorded using the pressure reading technique (Extech instruments, Waltham, USA) of Theodorou et al. (1994) while the CH_4 production was recorded using Gas-Pro detector (Gas Analyzer CROWCON Model Tetra3, Abingdon, UK).

After 48 h of incubation, bottles were uncapped, pH was measured using a pH meter and the contents of each bottle were filtered to obtain the non-fermented residue for determination of degraded substrate.

Table 1. Ingredients and chemical composition (g/kg DM) of total mixed rations of different CP concentrations.

	LCP	HCP
Ingredients		
Ground corn grain	302	228
Ground sorghum grain	280	280
Soybean meal	113	187
Corn stover	250	250
Cane molasses	30	30
Minerals[a]	25	25
Chemical composition		
Organic matter	935	931
Crude protein ($N \times 6.25$)	130	157
Neutral detergent fibre	367	355
Acid detergent fibre	139	140
Hemicellulose	228	215
Metabolizable energy (Mcal/kg)[b]	2.68	2.51

Note: LCP: low crude protein; HCP: high crude protein.
[a]Minerals/vitamins premix: Vitamin A (12 000 000 IU), Vitamin D_3 (2 500 000 IU), Vitamin E (15 000 IU), Vitamin K (2.0 g), Vitamin B_1 (2.25 g), Vitamin B_2 (7.5 g), Vitamin B_6 (3.5 g), Vitamin B_{12} (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).
[b]Calculated according to NRC (2001).

2.3. Degradability and sample analysis

Degradability and analysis were determined as it was descripted in Elghandour et al. (2014). Briefly, after 48 h of incubation, the fermentation process was stopped, where the contents of each serum bottle were filtered under vacuum through glass crucibles with a sintered filter. The obtained fermentation residues were dried at 105°C overnight to estimate DM disappearance. Both of NDF and acid detergent fibre (ADF) were determined in the residues after DM degradability (DMD) determinations for determining the degradability of NDF (NDFD) and ADF (ADFD). Samples of the feeds were analysed for DM (#934.01), ash (#942.05), N (#954.01), and EE (#920.39) according to AOAC (1997). The NDF and ADF content of both feeds and fermentation residues were determined using an ANKOM200 Fibre Analyzer Unit (ANKOM Technology Corp., Macedon, NY, USA) without use of an alpha amylase but with sodium sulfite in the NDF (Van Soest et al. 1991). Both NDF and ADF are expressed without residual ash.

2.4. Calculations and statistical analyses

As descripted before in Salem, Kholif, Elghandour, Hernandez et al. (2014), to estimate kinetic parameters of GP, results (ml/g DM) were fitted using the NLIN option of SAS (2002) according to France et al. (2000) model as:

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

where A is the volume of GP at time t, b is the asymptotic GP (ml/g DM), c is the rate of GP (/h), and L (h) is the discrete lag time prior to GP.

Metabolizable energy (ME, MJ/kg DM) and *in vitro* organic matter digestibility (OMD, g/kg OM) were estimated according to Menke et al. (1979):

$$ME = 2.20 + 0.136GP(ml/0.5gDM) + 0.057CP(g/kg\ DM)$$

$$OMD = 148.8 + 8.89GP + 4.5CP(g/kg\ DM) + 0.651ash(g/kg\ DM),$$

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

The partitioning factor at 24 h of incubation (PF_{24}; a measure of fermentation efficiency) was calculated as the ratio of DMD in vitro (mg) to the volume (ml) of GP at 24 h (i.e. DMD/total GP (GP_{24})) according to Blümmel et al. (1997). Gas yield (GY_{24}) was calculated as the volume of gas (ml gas/g DM) produced after 24 h of incubation divided by the amount of DMD (g) as:

$$\text{Gas yields } (GY_{24}) = \text{ml gas per g DM/g DMD.}$$

The experimental design for the *in vitro* ruminal GP, CH_4 production, degradability and fermentation parameters analysis was a completely random design considering, as fixed factors, type of ration (*R*) and yeast culture doses (*D*) in the linear model (Steel et al. 1997) within each yeast culture (*P*). Data of each of the three runs within the same sample were averaged prior to statistical analysis. Mean values of each individual sample within each species (three samples of each) were used as the experimental unit. The statistical model was

$$Y_{ijkl} = \mu + R_i + D_j + P_k + (R*D)_{ij} + (R*P)_{ik} + (P*D)_{jk} + (R*P*D)_{ijk} + E_{ijkl},$$

where Y_{ijkl} = is every observation of the *i*th ration (R_i) when incubated in the *j*th level D_j; μ is the general mean; R_i (i = 1–2) is the ration effect; D_j is the yeast doses effect (j = 1–3); P_k is the culture type (j = 1–3), $(R*D)_{ij}$ is the interaction between ration and yeast doses; $(R*P)_{jk}$ is the interaction between ration and culture; $(R*P*D)_{ijk}$ is the interaction between rations, yeast cultures and doses; and E_{ijkl} is experimental error. Linear and quadratic polynomial contrasts were used to examine responses of substrate to increasing addition doses of the yeast cultures.

3. Results

3.1. Gas and methane productions

Interactions were observed (P < .05) between ration × yeast culture, yeast culture × yeast dose, and between ration × yeast culture × yeast dose for gas and CH_4 productions. Compared to the LCP and without yeast addition (control treatments), HCP had increased (P = .001) asymptotic GP and decreased lag time (P < .001) without affecting the rate of GP (P > .05). Compared to control treatments, Biocell F53® and Biosaf SC47® increased the asymptotic GP (P < .001) in both rations where the dose of 2 mg yeast/g DM substrate was more effective (linear effect, P = .001; quadratic effect, P = .023) than the dose of 4 mg yeast/g DM substrate with the HCP ration. Regarding the LCP ration, the dose of 4 mg yeast/g DM substrate was more effective (linear effect, P = .001; quadratic effect, P = .023) to increase the asymptotic GP in both Biocell F53® and Procreatin 7® than the low dose (Table 2).

Increased CH_4 production was observed (P < .001) with the LCP ration than HCP ration after 24 and 48 h of incubation. For the HCP ration, the highest CH_4 productions at 24 h of incubation were observed (P < .01) with Procreatin 7® (at 2 mg/g DM) and with Biosaf SC47® (at 2, and 4 mg/g DM), while at 48 h of incubation was observed with the Procreatin 7® at 4 mg/g DM (P < .001). For the LCP ration, Biocell F53® and Biosaf SC47® had the highest CH_4 production at 24 h of incubation (P < .01), while at 48 h of incubation the dose of 4 mg/g DM of all tested yeast cultures decreased (P < .01) CH_4 at 48 h of incubation (Table 2).

3.2. Nutrients degradability and fermentation kinetics

Interactions between ration × yeast culture, yeast culture × yeast dose, and ration × yeast culture × yeast dose were observed (P < .05) for ME, PF_{24}, MCP, GY_{24}, DMD and OMD. The HCP ration had increased (P < .05) ME, MCP, GY_{24}, DMD and OMD with decreased PF_{24} compared to the LCP ration. Yeast culture had no effect on fermentation kinetics and nutrients degradability with exception of DMD (P < .001). With the HCP ration, addition of Procreatin 7® at 2 mg/g DM had increased ME, MCP, GY_{24}, DMD and OMD with decreased PF_{24} compared to the other doses of other yeast cultures. On the contrary and with the LCP ration, the dose of 2 mg/g DM from the culture Biocell F53® had increased ME, MCP, GY_{24}, and OMD compared to other doses of different yeast cultures; however, the dose of 2 mg/g DM of Procreatin 7® had increased DMD compared to other doses of other yeast cultures. No effect was observed (P > .05) on fermentation pH, NDFD and ADFD between the two rations, different yeast cultures and different yeast doses (Table 3).

4. Discussion

4.1. Gas production

Increasing protein content of the ration caused an increased GP. However, fermentability of protein produces relatively small GP compared to carbohydrate fermentation (Makkar et al. 1995). The GP, form any substrate, depends mainly on nutrient availability for rumen microorganisms (Elghandour et al. 2014; Elghandour, Kholif et al. 2015). Fermentation of dietary carbohydrates to acetate, propionate and butyrate produces gases (mainly CH_4, CO_2, H_2) in the rumen. However, in the current study, both rations (i.e. LCP and HCP) had almost the same fibre fractions content. So, it is well clear that the increased GP was a result of increased CP content. It is well known that SC has the ability to decrease ammonia production in the rumen (Hristov et al. 2013) by decreased protein degradation and decreased the overall N excretion by the animal, which would contribute to decreased ammonia emissions from cattle manure (Mao et al. 2013). The direct result of this action was the expected increased protein bypass in the rumen to be absorbed and metabolized as a true protein in the true stomach and small intestine.

Table 2. *In vitro* rumen gas and CH_4 production during 48 h of rumen incubation of two mixed rations as affected by different levels of three commercial *SC* cultures (mg/g DM).

			GP parameters			*In vitro* GP (mL/g DM) at:								CH_4 (mL/g DM) at:	
Ration	Yeast culture	Yeast dose (mg/g DM)	*b* (mL/g DM)	*c* (/h)	*L* (h)	2 h	4 h	6 h	8 h	10 h	12 h	24 h	48 h	24 h	48 h
HCP	Control	SC0	359.5	0.031	0.72	20.9	40.6	59.1	76.5	92.9	108.4	183.6	272.6	27.0	57.3
	Biocell® F53	SC2	409.6	0.026	0.40	20.7	40.2	58.8	76.5	93.2	109.0	188.6	289.3	38.8	66.6
		SC4	398.1	0.028	0.92	21.4	41.6	60.7	78.8	95.9	112.0	192.1	290.6	34.2	68.2
	Procreatin 7®	SC2	312.6	0.052	0.55	31.2	59.2	84.3	106.9	127.3	145.5	222.6	285.7	42.0	53.8
		SC4	309.8	0.051	0.49	30.3	57.6	82.3	104.6	124.6	142.7	219.7	283.5	23.6	96.1
	Biosaf® SC47	SC2	428.8	0.024	1.14	19.8	38.8	56.8	74.0	90.4	106.0	185.5	290.2	40.3	54.9
		SC4	401.3	0.026	0.57	20.2	39.4	57.6	74.8	91.2	106.8	184.8	283.9	36.5	64.2
LCP	Control	SC0	309.2	0.032	1.47	19.3	37.3	54.1	69.9	84.7	98.5	164.9	240.7	58.0	141.1
	Biocell® F53	SC2	362.1	0.042	1.16	29.0	55.7	80.2	102.7	123.4	142.4	228.3	311.9	143.2	164.6
		SC4	377.7	0.019	1.52	14.0	27.5	40.4	52.9	64.9	76.5	137.3	224.3	63.8	112.1
	Procreatin 7®	SC2	318.5	0.033	1.02	20.4	39.3	57.0	73.4	88.7	103.0	170.5	245.7	50.6	198.9
		SC4	393.6	0.017	1.35	12.9	25.4	37.4	49.1	60.4	71.2	129.3	215.2	48.5	53.4
	Biosaf® SC47	SC2	374.5	0.034	1.35	24.1	46.4	67.2	86.6	104.6	121.4	200.7	288.6	69.8	146.5
		SC4	328.1	0.050	1.39	30.8	58.7	83.9	106.7	127.4	146.0	226.4	295.8	69.9	108.8
	Pooled SEM		13.84	0.0035	0.283	2.21	4.11	5.74	7.14	8.33	9.33	12.56	12.44	7.51	9.94
	P value														
	Ration (*R*):		0.001	0.656	<0.001	0.071	0.060	0.050	0.041	0.034	0.027	0.006	<0.001	<0.001	<0.001
	Yeast culture (*P*):		<0.001	0.001	0.334	0.788	0.856	0.903	0.921	0.906	0.855	0.213	<0.001	<0.001	<0.001
	Yeast dose (*D*):														
	Linear		0.001	0.957	0.750	0.241	0.252	0.263	0.273	0.282	0.291	0.319	0.219	0.411	<0.001
	Quadratic		0.023	0.050	0.363	0.005	0.004	0.004	0.004	0.003	0.003	0.002	0.001	<0.001	0.653
	$R \times P$		<0.001	<0.001	0.962	<0.001	0.001	0.001	0.001	0.001	0.001	0.006	0.587	0.001	<0.001
	$R \times D$		0.020	0.069	0.625	0.126	0.116	0.107	0.100	0.093	0.087	0.062	0.057	0.082	0.118
	$P \times D$		0.002	0.001	0.441	0.006	0.006	0.006	0.006	0.006	0.007	0.009	0.025	<0.001	<0.001
	$R \times P \times D$		0.194	<0.001	0.663	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.001	<0.001	<0.001

Note: *b*: asymptotic gas production; *c*: rate of gas production; HCP: high crude protein; *L*: the initial delay before gas production begins; LCP: low crude protein. Means with different superscripts within each column are differ ($P < 0.05$).

Table 3. *In vitro* fermentation kinetic and degradabilities of two mixed rations as affected by different levels of three commercial *SC* cultures (mg/g DM).

			Fermentation kinetic					Nutrients degradabilities			
Ration	Yeast culture	Yeast dose (mg/g DM)	pH	ME	PF_{24}	MCP	GY_{24}	DMD	OMD	NDFD	ADFD
HCP	Control	SC0	6.71	8.09	5.60	619.3	178.7	585.8	550.4	327.5	224.8
	Biocell® F53	SC2	6.77	8.22	5.54	628.6	180.6	317.0	559.2	279.0	201.0
		SC4	6.82	8.32	5.51	635.2	181.4	646.0	565.5	381.7	231.3
	Procreatin 7®	SC2	6.75	9.15	5.33	692.2	187.7	608.3	619.7	323.0	226.7
		SC4	6.74	9.07	5.33	686.8	187.7	721.0	614.5	326.3	224.7
	Biosaf® SC47	SC2	6.85	8.14	5.56	622.9	179.8	633.7	553.8	324.7	225.3
		SC4	6.74	8.12	5.57	621.6	179.6	670.3	552.6	332.7	198.3
LCP	Control	SC0	6.90	7.43	5.76	584.5	173.7	511.6	504.8	346.5	222.5
	Biocell® F53	SC2	6.96	9.15	5.29	702.9	189.0	248.7	617.5	344.3	222.3
		SC4	6.93	6.68	6.08	532.8	164.4	491.3	455.7	339.3	225.3
	Procreatin 7®	SC2	6.86	7.58	5.73	594.9	174.8	582.0	514.7	321.7	221.3
		SC4	6.76	6.46	6.21	517.7	161.1	349.0	441.4	327.3	225.7
	Biosaf® SC47	SC2	6.88	8.40	5.50	651.3	182.3	422.7	568.4	300.7	211.7
		SC4	6.65	9.10	5.29	699.5	189.0	578.0	614.2	332.0	228.3
	Pooled SEM		0.473	0.342	0.342	0.105	23.50	53.78	22.34	29.02	10.78
	P value										
	Ration (*R*):		0.597	0.001	0.001	0.001	0.006	<0.001	0.001	0.656	0.659
	Yeast culture (*P*):		0.473	0.213	0.213	0.065	0.214	<0.001	0.213	0.997	0.758
	Yeast dose (*D*):										
	Linear		0.273	0.319	0.319	0.787	0.319	0.387	0.319	0.864	0.832
	Quadratic		0.350	0.002	0.002	0.002	0.002	0.001	0.002	0.123	0.370
	$R \times P$		0.462	0.006	0.006	0.015	0.006	0.072	0.006	0.764	0.903
	$R \times D$		0.524	0.062	0.062	0.019	0.062	0.095	0.062	0.578	0.681
	$P \times D$		0.272	0.009	0.009	0.005	0.009	0.002	0.009	0.640	0.492
	$R \times P \times D$		0.336	<0.001	<0.001	<0.001	<0.001	0.004	<0.001	0.530	0.227

Notes: DMD: *in vitro* dry matter disappearance; GY_{24}: gas yield at 24 h of incubation; HCP: high crude protein; LCP: low crude protein; MCP: microbial crude protein production; ME: metabolizable energy; OMD: *in vitro* organic matter digestibility; PF_{24}: partitioning factor at 24 h of incubation. Means with different superscripts within each column are differ ($P < 0.05$).

Decreased lag time with increased protein content (i.e. HCP ration) reflects the fast activity of SC on the fermentation process. Newbold et al. (1996) stated that SC can affect the respiratory activity that scavenges O_2 (Chaucheyras-Durand et al. 2008), which is toxic to anaerobic bacteria and causes inhibition of adhesion of cellulolytic bacteria to cellulose, and this peak in O_2 concentration occurs at approximately the time of feeding (i.e. initial time). Moreover, SC contains small peptides and other nutrients that required to predominant ruminal cellulolytic bacteria to initiate growth (Callaway and Martin 1997). Activity of SC depends on many factors including availability of nutrients for rumen microorganisms will stimulate fermentation process (Paya et al. 2007). Previous studies reported that the stimulation of cellulose degradation by SC addition was associated with a decreased lag time, which results in increased initial rates of digestion, but not in increased extent of digestion by ruminal microorganisms (Williams et al. 1991).

Both of Biocell F53® and Biosaf SC47® cultures improved GP than Procreatin 7®. This may be related with the nature of each culture and their contents of live cells, and other nutrients/carrier materials.

The low dose of SC used (SC2) improved GP than the high dose (SC4). However, many reports stated an increased GP with increasing SC dose (Mao et al. 2013; Elghandour et al. 2014). The nature of substrate, and the *in vitro* procedure are responsible about the varied response with a different level of SC. In case of *in vitro* technique, the substrate amount relative to the used rumen liquid volume for incubation is much less than in the rumen of a cow (<1 *vs* 12%). In case of rumen modulator such as SC supplementation at different rates, SC could change the fermentation rate and cause different substrate depletion, resulting in different responses (Mao et al. 2013).

4.2. CH_4 production

Before the first 24 h of incubation, CH_4 production was negligible and then started to be increased quickly to reach its concentration peak at the end of incubation; however, GP started early with incubation. This reflects the nature of the produced gases. During fermentation process, amounts of gases are produced within the rumen, which mainly constitutes H_2, CO_2 and CH_4. As previously mentioned, increasing ration CP content caused an increased GP with decreasing CH_4 production. This result might be due to an increased proportion of protein in the ration, which changes the produced short chain fatty acids concentrations in such a way that less acetic and more propionic is formed, and hence, the supply of hydrogen for methanogenesis is limited (Polyorach et al. 2014) with reducing the protozoal population (Iqbal et al. 2008).

CH_4 production differed between yeast cultures. This may be illustrated based on different cultures contents from other components such as CP, crude fibre, crude fat ash, and/or materials of coating.

Moreover, the low dose of SC (SC2) increased CH_4 production than the SC4. This related with the increased GP with this dose of SC and the changed nature of produced gas due to SC addition. Elghandour et al. (2014) noted an increased CH_4 production as the produced gases was increased when SC was added. However, increasing the dose of SC decreased CH_4 production. Some studies suggested that SC culture might stimulate the acetogens to compete or to co-metabolize H_2 with methanogens, thereby reducing CH_4 productions (Mwenya et al. 2004; Elghandour et al. 2014). Polyorach et al. (2014) noted that CH_4 production in the rumen was decreased when animals fed SC fermented cassava chip protein instead of

soybean meal. They returned it to the ability of SC to affect H_2 metabolism in the rumen with altering the fermentation process in a manner that reduces the formation of CH_4. However, other studies (Martin and Nisbet 1992) reported an increased CH_4 production. These conflicting results on CH_4 production are likely due to strain difference of SC cultures and nature of rations (Patra 2012).

4.3. pH and nutrient degradabilities

Ruminal pH was not affected during fermentation processes. Several studies have suggested that SC moderate the ruminal pH by increasing lactate utilization making pH relatively more stable and meet the needs of rumen microbes to perform its activity (Elghandour et al. 2014).

Nutrients degradability showed an improved DMD and OMD without affecting on NDFD and ADFD with HCP even with SC addition. However, different SC cultures affected only on DMD. Both of rations had a much closed fibre fractions contents with different CP content. So, the improved DMD and OMD were a result of increased CP, which improved the microflora activity in the rumen. These could be due to increased protein level that would provide more readily available energy, enhancing corresponding of microbes due to the better supply of fermentable OM, energy and nitrogen to rumen bacteria, consequently, increased degradability (Polyorach et al. 2014). Bach et al. (2005) indicated that the most important factors affecting utilization of dietary protein in the rumen included type of protein, carbohydrate and their interactions and the predominant microbial population in the rumen. The unaffected NDFD and ADFD with changing protein content; however, SC was added, which may be due to the high protein content of the ration.

It is well known that SC had the ability to stimulate growth and activity of total ruminal anaerobes bacteria (Jouany 2001). Polyorach et al. (2014) showed that SC can increase rumen microorganism's total numbers and improve the utilization of feeds. However, most of the reports showed an improved fibres fractions' degradability (Elghandour et al. 2014) as a result of increased cellulolytic digester species *Fibrobacter succinogenes, Ruminococcus flavifaciens* and *Selenomonas ruminantium* (Callaway and Martin 1997). Guedes et al. (2008) stated unaffected fibre fractions with addition of SC.

4.4. In vitro *rumen fermentation kinetic*

Improved ME, MCP, and GY_{24} were observed with the HCP ration. Rations with high protein content provide ruminal microflora with the essential nutrients for its activity. The highly activity reflected on higher GP, higher microbial protein synthesis, and higher degradability. This can be generalized for the effect of SC addition on the fermentation activity. Mao et al. (2013) and Elghandour et al. (2014) showed that addition of SC increased ME. They returned their results to the high activities of microbes in the rumen as a result of produced growth factors for microbial growth and activity in the rumen, and to the ability of SC to provide conducive anaerobic conditions to microbial growth (Mosoni et al. 2007).

Result of PF_{24} reflects decreased conversion of degraded substrate into microbial biomass (Harikrishna et al. 2012). Elghandour et al. (2014) showed that addition of SC decreased PF from different poor-quality roughages.

5. Conclusions

The high CP rations increased GP and decreased CH_4 production *versus* the low CP ration. Addition of *S. cerevisiae* improved ruminal fermentation kinetics with reducing CH_4 production. The commercial *S. cerevisiae* cultures of Biocell F53® and Biosaf SC47® addition at rate of 2 mg/g DM improved fermentation kinetics and nutrients' degradability.

Disclosure statement

No potential conflict of interest was reported by the authors.

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