PAPER

Influence of live cells or cells extract of *Saccharomyces cerevisiae* on *in vitro* gas production of a total mixed ration

Miguel Pulido Rodriguez,[1] María D. Mariezcurrena,[2] María A. Mariezcurrena,[1] Blas Cruz Lagunas,[1] Mona M.M.Y. Elghandour,[1] AbdelKader M. Kholif,[3] Ahmed E. Kholif,[3] Ernesto Morales Almaráz,[1] Abdelfattah Z.M. Salem[1]

[1]Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma del Estado de México, Toluca, Mexico
[2]Facultad de Ciencias Agrícolas, Universidad Autónoma del Estado de México, Toluca, Mexico
[3]Dairy Science Department, National Research Centre, Giza, Egypt

Abstract

The effect of *Saccharomyces cerevisiae* as live cells (LC) or cells extract (CE) on *in vitro* gas production (GP) kinetics and ruminal fermentation parameters of a total mixed ration (TMR) consisting of commercial concentrate and alfalfa hay [1:1 dry matter (DM)] as a substrate was studied. The TMR was incubated with CE at 1, 2 and 4 mg/g or LC at 0.3, 0.6 and 0.9 mg/g DM for 96 h. Rumen GP was recorded after 6, 12, 19, 24, 48, 72 and 96 h of incubation. Interaction effects were observed (P<0.01) between treatment type and yeast dose for the asymptotic GP and methane (CH_4) production. Incubation of yeast CE improved (P<0.01) the asymptotic GP compared to control and LC with greater effects (P<0.01) for the low and the intermediate doses. Yeast CE treatment was more effective (P<0.01) in GP than both of LC and control treatments with greater effect (P<0.01) for the low and the intermediate doses. Treatment type and yeast dose affected (P<0.01) CH_4 production, metabolisable energy (ME), and short chain fatty acids (SCFA) without affecting *in vitro* DM degradability (IVDMD). Higher values (P<0.01) of CH_4, ME, SCFA and IVDMD were observed for the yeast CE treatment. It could be concluded that adding yeast *S. cerevisiae* (CE and LC extract) improved GP and ruminal fermentation parameters, where CE at 0.3 and 0.6 mg/g DM was more effective than the yeast LC.

Introduction

In ruminant species, there is a possibility for losing energy and protein due to ruminal fermentation processes (Ando *et al.,* 2004; Salem *et al.*, 2014a). Ionophores and antibiotics were good strategies for reducing energy and protein losing for many years (McGuffey *et al.*, 2001). However, the European Union banned their use due to the potential appearance of residues in milk and meat (Russell and Houlihan, 2003). For this reason, there is substantial interest to evaluate the potential of using natural feed additives, generally recognized as safe for human consumption, to modify rumen microbial fermentation and improve feed utilization (Chaucheyras-Durand *et al.*, 2008). Phytogenic extracts (Salem *et al.*, 2014b; Cedillo *et al.,* 2014, 2015), fibrolytic enzymes (Togtokhbayar *et al.,* 2015; Valdes *et al.*, 2015) and yeast (Elghandour *et al.,* 2014) proved to be good strategies to modulate ruminal fermentation for better feed utilization. Yeast *Saccharomyces cerevisiae*, live cells (LC) or cells extract (CE) are generally recognised as safe by the US Food and Drug Administration, and they can be legally used as animal feed additives (Kwiatkowski and Kwiatkowski, 2012).

Yeast is a natural feed additive used to promote growth and activity of rumen microbes through stabilising rumen fermentation and preventing rumen flora disorders and disturbances (Kumar *et al.*, 2013; Pinloche *et al.*, 2013). Increased viable bacterial numbers (Jouany, 2001) as a result of yeast supplementation with enhanced ammonia utilization by ruminal microorganisms (Chaucheyras-Durand *et al.*, 2008) were achieved. Elghandour *et al.* (2014) reported an increased *in vitro* rumen degradability of forages as a result of stimulated growth and activity of fibrolytic bacteria due to yeast addition. Yeast CE is a non-antibiotic functional product that is naturally obtained from yeast. Although the composition of yeast extracts is variable, it contains three major constituents: glucan (glucose polysaccharide), mannan (mannose polysaccharide) and a protein fraction (Kwiatkowski and Kwiatkowski, 2012). The content of yeast CE from glucan vary from 1% (Lille and Pringle, 1980) to about 29% (Sedmak, 2006) of the dry weight. The content depends upon the nutritional status of yeast cells, isolation method, analysis method and the phase of growth at cells harvest (Lille and Pringle, 1980; Kwiatkowski and Kwiatkowski, 2012).

Mannan and glucan are not degradable in the rumen by animal's digestive enzymes; so, they pass through the digestive tract with the pathogens attached thus prevent the colonization of the pathogenic bacteria (Wellens *et al.*, 2008). Mannan and glucan addition has been shown to increase both intestinal and serum IgG levels in dairy cows and calves (Franklin *et al.*, 2005).

Therefore, the current study aimed to study the effect of yeast, as feed additive; in two different forms as LC or CE in the *in vitro* ruminal fermentation and gas production (GP) of a total mixed ration (TMR) consisted from commercial concentrate and hay alfalfa hay [1:1 dry matter (DM).

Corresponding author: Dr. Abdelfattah Z.M. Salem, Facultad de Veterinaria y Zootecnia, Universidad Autónoma del Estado de México, Instituto literario N. 100, C.P. 50000, Col. Centro, Toluca, Estado de México, México.
Tel. +521.722.2965542 - Fax: +521.722.1806194.
E-mail: asalem70@yahoo.com

Key words: Gas production; Rumen fermentation; Yeast cells extract; Yeast live cell.

Received for publication: 23 October 2014.
Accepted for publication: 25 January 2015.

Italian Journal of Animal Science 2015; 14:3713
doi:10.4081/ijas.2015.3713

Materials and methods

Yeast live cells and yeast cells extract products

Two commercial yeast products of yeast cells extract and live yeast cells were used. The product of live cells Selyeast3000® (Biosaf SC47®; Lesaffre Feed Additives, Toluca, Mexico) is a highly concentrated source of organic selenium to be used in animal feed. The product contains 47.0% crude protein (CP), 0.3% selenium and 5% moisture. The product also characterized by its content from total coliforms less than 100 CFU/g with no Salmonella. The product of yeast cell extract Safmannan® (Biosaf SC47®) is a highly concentrated source of mannan oligosaccharide and ß-glucanos derived from a primer inactivated yeast (*S. cerevisiae*) for use in animal

feed. The product contains 14% CP, 20% fat, 24% ß-glucans, 22% mannose and less than 6% ash. Yeast CE at 1, 2 and 4 mg/g DM and yeast LC at 0.3, 0.6 and 0.9 mg/g DM were tested throughout 96 h of incubation.

In vitro incubations

Rumen inoculum was obtained from two Brown Swiss cows (400-450 kg body weight) fitted with permanent rumen cannula and fed *ad libitum* with a TMR consisting of commercial concentrate 1:1 DM (PURINA®, Toluca, Mexico) and alfalfa hay (Table 1) formulated to meet nutrient requirements (National Research Council, 2001). Fresh water was available for cows at all times during the collection phase of ruminal inoculum.

Rumen contents of each cow were obtained before the morning meal, mix and filtered through four layers of cheesecloth into a flask with O_2 headspace. Samples of TMR were weighed into 120 mL serum bottles with appropriate addition of *S. cerevisiae* LC or CE dose/g DM. Accordingly, 10 mL particle free ruminal fluid was added to each bottle followed by 40 mL of the buffer solution according to Goering and Van Soest (1970), without added trypticase, in (v/v) ratio of 1: 4.

A total of 162 bottles [3 bottles of each doses in three different runs for each of the treatments with three bottles as blanks (rumen fluid only)] were used. Once all the bottles filled, they were immediately closed with rubber stoppers, shaken and placed in the incubator at 39°C. Gas production readings were made at 6, 12, 19, 24, 48, 72, and 96 h post inoculation, using the technique of pressure reading (Extech Instruments, Waltham, USA) of Theodorou *et al.* (1994). At the end of incubation (*i.e.*, 96 h), bottles were uncapped, the pH was measured using a pH meter (Conductronic pH15, Puebla, Mexico). Contents of each bottle were then transferred to filter the residue for determination of apparent degraded substrate.

After recording the final gas volume at 96 h of incubation, 2 mL of NaOH (10 M) were added to each bottles and gas pressure was determined immediately. Mixing of the contents with NaOH allowed absorption of carbon dioxide, with the gas volume remaining in the headspace of bottles considered to be CH_4 (Demeyer *et al.*, 1988).

Dry matter degradability and sample analysis

The DM degradability was determined as descripted previously in Elghandour *et al.* (2014). In brief, at the end of incubation (*i.e.*, 96 h), the contents of each serum bottle were filtered under vacuum through glass crucibles with a sintered filter (coarse porosity no. 1, pore size 100–160 m, Pyrex, Stone, UK). Fermentation residues were dried at 65°C overnight to estimate DM disappearance. Loss in weight after drying being the measure of non-degradable DM. Dry matter degradability (mg/g DM) at 96 h of incubation was calculated as the difference between DM content of substrate and its non-degradable DM (Ørskov and McDonald, 1979).

Samples of the feeds were analysed for DM (ID 934.01), ash (ID 942.05), N (ID 954.01) and ether extract (ID 920.39) according to AOAC (1997). The neutral detergent fibre (NDF; Van Soest *et al*., 1991) and acid detergent fibre (ADF) content of both feeds and fermentation residues were determined using an ANKOM200 Fibre Analyser Unit (ANKOM Technology Corp., Macedon, NY, USA) without use of an alpha amylase but with sodium sulfite in the NDF. Both NDF and ADF are expressed without residual ash.

Calculations and statistical analyses

All the calculations were mentioned previously in Elghandour *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) 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) was estimated according to Menke *et al.* (1979) as:

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

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

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

$$SCFA\ (mmol/200\ mg\ DM)=0.0222\ GP-0.00425$$

where GP is the 24 h net GP (mL/200 mg DM).

Data on *in vitro* ruminal fermentation parameters, GP parameters, *in vitro* DM degradability (IVDMD) and ME were analysed as 2 × 3 factorial arrangement [2 additives (LC and CE) × 3 levels (LC 1, 2 and 4 mg/g DM, CE 0.3, 0.6 and 0.9 mg/g of DM and Control 0 mg/g of DM)] with three repetitions (Steel and Torrie, 1980). The mixed model was:

$$Y_{jkl}=\mu+Sp_j+EL_k+Sp_j*EL_k+E_{jkl}$$

where Y_{jkl} represents response variables (ruminal fermentation activity, energy utilization of metabolizable energy and short chain fatty acids) for the (j) additives and (k) level; μ = general mean; Sp_j = effect of j- additive; EL_k = effect of k- level; $Sp_j * EL_k$ = interaction of the j- additives with k- level; E_{jkl} = the error term–NI (0, 2). In the case of significant ($P<0.05$) interactions, Tukey test was used to separate means additives (Steel and Torrie, 1980).

Results

In vitro gas production

An interaction effect was observed ($P=0.009$) between treatment type and treatment dose for the asymptotic GP without interaction effects ($P>0.05$) for the rate of GP and the initial delay before gas production begins. In general, incubation of yeast CE improved ($P=0.0007$) the asymptotic GP compared to control and yeast LC. In both of CE and LC, the low and the intermediate doses were more

Table 1. Ingredients and chemical composition of the incubated total mixed ration.

	TMR
Ingredients, g/kg DM	
Sorghum grain	195
Corn grain	195
Soybean meal	160
Broilers wests	140
Corn stover	110
Orange peel	100
Wheat bran	80
Vitamins and minerals°	20
Chemical composition, g/kg DM	
Dry matter	883.2
Organic matter	958.1
Crude protein	158.4
Ether extract	31.7
Neutral detergent fibres	227.0
Acid detergent fibres	140.5

TMR, total mixed ration; DM, dry matter. °Mineral and vitamin premix (OVISALT): Ca, 18.00%; P, 0.02%; Mg, 1.79%; Zn, 4066.19 ppm; Mn, 3168.48; ppm; Fe, 2338.98 ppm; Cu, 12.62 ppm; I, 40.17 ppm; Se, 41.48 ppm; Co, 18.60 ppm; Vitamin A, 150,000.00 U/kg; Vitamin D, 25,000.00 U/kg; Vitamin E, 150.00 U/kg.

effective (P=0.009) than the higher dose (Table 2).

Before the first 12 h of incubation, no effects for both of treatment type and treatment dose on the GP. However, after 12 h of incubation, treatment affected (P<0.01) the GP. After 19 h of incubation, the treatment dose affected (P<0.01) GP. Yeast CE had greater effect (P<0.01) in GP than both of yeast LC and control treatment. In both yeast types, the low and the intermediate doses had greater effects (P<0.01) in GP than the higher dose (Table 2).

In vitro ruminal fermentation parameters

No treatment and dose interaction effect (P<0.05) was observed for pH. However, an interaction effect was observed (P<0.01) for CH_4 production. In the contrary, both of treatment and dose insignificantly (P>0.05) affected the pH values. However, treatment type and treatment dose affected (P<0.01) CH_4, ME, and SCFA without affecting IVDMD. No differences (P>0.05) were observed between the control and yeast LC treatments for CH_4 production (at 96 h), ME, SCFA and IVDMD. However, higher values (P=0.001) were observed for the yeast CE treatment for the previous parameters (Table 3). Regarding the dose effect, the most effective dose was the intermediate dose for yeast CE and the low dose for yeast LC treatment (Table 3).

Discussion

In vitro gas production

The obtained result of GP showed improved GP as a result of yeast addition. Increased GP with addition of yeast was showed in many studies (Tang *et al.*, 2008; Elghandour *et al.*, 2014). This may have resulted from the increased production of propionate fatty acid due to improved ruminal fermentation. Because carbon dioxide is produced when propionate is made by some ruminal bacteria via the succinate:propionate pathway (Wolin and Miller, 1988). Fermentation of dietary carbohydrates to volatile fatty acids produces gases in the rumen, which mainly constitutes hydro-

Table 2. Impact of live cells and cell extract of *Saccharomyces cerevisiae* on *in vitro* gas production parameters, and gas volume accumulated after different hours of incubation.

		GP parameters			*In vitro* GP, mL/g DM						
	Yeast dosage, mg/g DM	*b*, mL/g DM	*c*, /h	*L*, h	GP_6	GP_{12}	GP_{19}	GP_{24}	GP_{48}	GP_{72}	GP_{96}
Control	0	176.3^{bc}	0.086	1.57	55.6	104.0^{ab}	136.5^{b}	150.3^{b}	172.9^{bc}	175.9^{bc}	176.2^{bc}
LC	1	182.1^{abc}	0.078	1.40	55.1	102.7^{ab}	136.1^{b}	151.0^{b}	177.3^{abc}	181.4^{abc}	182.0^{abc}
	2	176.5^{bc}	0.084	1.99	49.9	99.8^{b}	133.7^{b}	148.3^{b}	172.6^{bc}	176.0^{bc}	176.4^{bc}
	4	164.9^{c}	0.100	2.25	50.9	100.7^{b}	131.4^{b}	143.6^{b}	162.3^{c}	164.6^{c}	164.9^{c}
CE	0.3	195.3^{a}	0.087	1.26	65.8	118.2^{a}	153.2^{a}	168.0^{a}	191.9^{a}	194.9^{a}	195.3^{a}
	0.6	195.7^{a}	0.087	1.24	66.5	119.1^{a}	154.1^{a}	168.8^{a}	192.4^{a}	195.3^{a}	195.6^{a}
	0.9	185.7^{ab}	0.082	1.22	60.3	109.1^{ab}	142.5^{ab}	157.1^{ab}	181.7^{ab}	185.1^{ab}	185.6^{ab}
Pooled SEM		3.97	0.0063	0.284	4.09	3.84	3.21	3.01	3.62	3.92	3.97
P value											
Treatment		0.0007	0.4722	0.1666	0.0769	0.0111	0.0005	0.0001	0.0003	0.0006	0.0007
Dose		0.0089	0.3859	0.4079	0.4374	0.1356	0.0149	0.0045	0.0054	0.0082	0.0086
Treatment×Dose		0.0089	0.3859	0.4079	0.153	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

GP, gas production; DM, dry matter; *b*, asymptotic gas production; *c*, rate of gas production; *L*, initial delay before gas production begins; LC, live cells; CE, cell extract; $^{a-c}$Different letters following means in the same column indicate differences at P<0.05.

Table 3. Impact of live cells and cell extract of *Saccharomyces cerevisiae* on ruminal fermentation parameters

	Yeast dosage, mg/g DM	pH	CH4, mL/ g DM	ME, MJ/kg DM	SCFA, mmol/g DM	IVDMD, mg/g DM
Control	0	6.71	2.20^{b}	11.30^{b}	1.33^{b}	823.7
LC	1	6.69	1.95^{b}	11.30^{b}	1.34^{b}	869.7
	2	6.72	2.02^{b}	11.20^{b}	1.31^{b}	878.0
	4	6.71	2.14^{b}	10.90^{b}	1.27^{b}	858.7
CE	0.3	6.72	1.96^{b}	12.23^{a}	1.49^{a}	855.6
	0.6	6.75	2.71^{a}	12.30^{a}	1.49^{a}	818.0
	0.9	6.75	2.16^{b}	11.63^{ab}	1.39^{ab}	754.0
Pooled SEM		0.030	0.069	0.161	0.026	27.84
P value						
Treatment		0.7902	0.0001	0.0001	0.0001	0.1107
Dose		0.8867	0.0001	0.0041	0.0046	0.2290
Treatment×Dose		0.8867	0.0001	0.0001	0.0001	0.241

pH, ruminal pH; CH_4, methane production; ME, metabolisable energy; SCFA, short-chain fatty acids; IVDMD, *in vitro* dry matter degradability; LC, live cells; CE, cell extract. $^{a-c}$Different letters following means in the same column indicate differences at P<0.05.

gen, carbon dioxide and CH_4. Addition of yeast not only has the ability to improve GP, but also, can make qualitative changes in produced gases making it less negatively affects environment (Hristov *et al.*, 2013).

Improved GP with yeast CE than LC reflects the enhanced incubation environment. A number of specific hypothetical biochemical mechanisms have been developed to explain the stimulatory effects of yeast in ruminal fermentation (Chevaux and Fabre, 2007). Some of these mechanisms have been based on the ability of yeast to provide important nutrients or nutritional cofactors that stimulate microbial activities in the rumen (Callaway and Martin, 1997). Yeast CE material is stable to acid digestion in the digestive tract and various fractions are known to survive passage through the stomach (Wellens *et al.*, 2008). The ability of yeast CE to remain unchanged through acid conditions found in the stomach and intestine that may account for the product's biological activity in a wide range of species. Moreover, the ability of yeast to scavenge excess oxygen, from rumen, creates a more optimal environment for rumen anaerobic bacteria (Newbold *et al.*, 1996; Jouany, 2001). In addition, *S. cerevisiae* supplementation could provide vitamins such as biotin and thiamine, which are reported to be required for microbial growth and activity (Callaway and Martin, 1997). Other studies suggested that yeast can provide a site for metabolic exchanges and a suitable environment that promotes the growth and activity of beneficial microorganisms around substrates (Jouany, 2001).

From obtained results, *S. cerevisiae* addition to the incubated diet decreased the lag time with increasing the asymptotic GP. Elghandour *et al.* (2014) illustrated this phenomenon based on two basic mechanisms. The first mode of yeast action reported by Newbold *et al.* (1996) is the respiratory activity that scavenges oxygen, which is toxic to anaerobic bacteria and causes inhibition of adhesion of cellulolytic bacteria to cellulose, and this peak in oxygen concentration occurs at approximately the time of feeding (*i.e.*, initial time). The second mode of action is that yeast contains small peptides and nutrients that required to ruminal cellulolytic bacteria to initiate microbial growth (Callaway and Martin, 1997). Ando *et al.* (2004) stated that the asymptotic GP was higher with the addition of yeast extract to Italian ryegrass and whole crop corn. However, they found that the values were greater with the addition of yeast than with the addition of yeast extract.

In vitro ruminal fermentation parameters

In previous studies, adding *S. cerevisiae* increased SCFA production and ME from forage substrates (Mao *et al.*, 2013; Elghandour *et al.*, 2014). Increased SCFA production and ME are associated with high activities of microbes in the rumen. *S. cerevisiae* produces growth factors for microbial growth that can stimulate rumen microbial growth and activity (Chiquette, 2009). Moreover, *S. cerevisiae* has ability to provide conducive conditions to microbial growth in a way that is capable of using oxygen in the rumen so that the conditions of an aerobic rumen awake (Mosoni *et al.*, 2007). Newbold *et al.* (1996), for example, used this mode of action to explain a 35% increase in total bacterial counts with *S. cerevisiae* addition *in vitro*. Increased SCFA is important in terms of enhanced lactose production, milk volume and overall energy balance (Khattab *et al.*, 2011; Kholif *et al.*, 2014, 2015).

Most of experiments studied the effect of *S. cerevisiae* on CH_4 production was *in vitro* (Elghandour *et al.*, 2014). In the current study, addition of yeast LC lowered the CH_4 production compared to the yeast cells extract. Some studies suggested that yeast might stimulate the acetogens to compete or to co-metabolize hydrogen with methanogens thereby, reducing CH_4 emissions (Mwenya *et al.*, 2004; Elghandour *et al.*, 2014). However, other studies increased CH_4 production (Martin *et al.*, 1989; Martin and Nisbet, 1992). These conflicting results of CH_4 production are likely due to yeast strain difference and type of diets.

Ruminal pH was not changed during fermentation processes. Several studies have suggested that *S. cerevisiae* cultures moderate the ruminal pH by increasing lactate utilization, making relatively more stable pH and meet the needs of rumen microbes to perform its activity (Chaucheyras-Durand *et al.*, 2008; Elghandour *et al.*, 2014).

Yeast could stimulate growth and activity of total ruminal anaerobes and cellulolytic bacteria (Girard, 1996; Jouany, 2001). According to Girard (1996), *S. cerevisiae* can increase rumen microorganism total numbers, improve fibre digestion, reduce lactate accumulation, reduce the concentration of oxygen in rumen fluid so as to improve utilisation of fed ration. Researchers have reported increases in the rate of cellulose digestion by *Fibrobacter succinogenes, Ruminococcus flavifaciens* and *Selenomonas ruminantium* in response to yeast supplementation (Callaway and Martin, 1997; Sullivan and Martin, 1999). Yeast has also directly stimulated rumen fungi, which may improve fibre digestion (Chaucheyras *et al.*, 1995). Ando *et al.* (2004) found that, in Italian ryegrass, whole crop corn and rice straw, higher degradability values were observed for the samples incubated with the yeast extract.

Conclusions

Administration of yeast *S. cerevisiae* to the diets in two different forms as a LC or CE was effective to improve GP and ruminal fermentation parameters than the control. Addition of yeast CE was more effective than the yeast LC. In both type of treatments, the low and intermediated doses were more effective than the higher doses.

References

Ando, S., Khan, R.I., Takahasi, J., Gamo, Y., Morikawa, R., Nishiguchi, Y., Hayasaka, K., 2004. Manipulation of rumen fermentation by yeast: the effect of dried beer yeast on the in vitro degradability of forages and methane production. Asian Austral. J. Anim. 17:68-72.

AOAC, 1997. Official methods of analysis, 16th ed. Association of Official Analytical Chemists, Arlington, VA, USA.

Callaway, E.S., Martin, S.A., 1997. Effects of a Saccharomyces cerevisiae culture on ruminal bacteria that utilize lactate and digest cellulose. J. Dairy Sci. 80:2035-2044.

Cedillo, J., Kholif, A.E., Salem, A.Z.M., Elghandour, M.M.Y., Vázquez, J.F., Alonso, M.U., Barbabosa, A., Chagoyán, J.C.V., Reyna, A.G., 2015. Oral administration of Sauce llor on extract to growing lambs to control gastrointestinal nematodes and Moniezia spp. Asian Pac. J. Trop. Med. 8:520-525.

Cedillo, J., Vázquez-Armijo, J.F., González-Reyna, A., Salem, A.Z.M., Kholif, A.E., Hernández-Meléndez, J., Martínez-González, J.C., de Oca Jiménez, R.M., Rivero, N., López, D., 2014. Effects of different doses of Salix babylonica extract on growth performance and diet in vitro gas production in Pelibuey growing lambs. Ital. J. Anim. Sci. 13:3165.

Chaucheyras, F., Fonty, G., Bertin, G., Gouet, P., 1995. In vitro utilization by a ruminal acetogenic bacterium cultivated alone or in

association with an Archea methanogen is stimulated by a probiotic strain of Saccharomyces cerevisiae. Appl. Environ. Microb. 61:3466.

Chaucheyras-Durand, F., Walker, N.D., Bach, A., 2008. Effects of active dry yeasts on the rumen microbial ecosystem: past, present and future. Anim. Feed Sci. Tech. 145:5-26.

Chevaux, E., Fabre, M.M., 2007. Probiotic yeast in small ruminants. Feed Mix 15:28-29.

Chiquette, J., 2009. The role of probiotics in promoting dairy production. Available from: http://www.wcds.ca/proc/2009/Manuscripts/RoleOfProbiotics.pdf

Demeyer, D., De Meulemeester, M., De Graeve, K., Gupta, B.W., 1988. Effect of fungal treatment on nutritive value of straw. Int. S. Crop 53:1811-1819.

Elghandour, M.M.Y., Vázquez Chagoyán, J.C., Salem, A.Z.M., Kholif, A.E., Martínez Castañeda, J.S., Camacho, L.M., Cerrillo-Soto, M.A., 2014. Effects of Saccharomyces cerevisiae at direct addition or pre-incubation on in vitro gas production kinetics and degradability of four fibrous feeds. Ital. J. Anim. Sci. 13:295-301.

France, J., Dijkstra, J., Dhanoa, M.S., López, S., Bannink, A., 2000. Estimating the extent of degradation of ruminant feeds from a description of their gas production profiles observed in vitro: derivation of models and other mathematical considerations. Brit. J. Nutr. 83:143-150.

Franklin, S.T., Newman, M.C., Newman, K.E., Meek, K.I., 2005. Immune parameters of dairy cows fed mannan oligosaccharide and subsequent transfer of immunity to calves. J. Dairy Sci. 88:766-775.

Getachew, G., Makkar, H.P.S., Becker, K., 2002. Tropical browses: contents of phenolic compounds, in vitro gas production and stoichiometric relationship between short chain fatty acid and in vitro gas production. J. Agr. Sci. 139:341-352.

Girard, I.D., 1996. Characterisation of stimulatory activities from Saccharomyces cerevisiae on the growth and activities of ruminal bacteria. PhD Diss., University of Kentucky, Lexington, KY, USA.

Goering, M.K., Van Soest, P.J., 1970. Forage fibre analysis (apparatus, reagents, procedures and some applications). Agricultural Research Service, USDA, Washington, DC, USA.

Hristov, A.N., Oh, J., Firkins, J.L., Dijkstra, J., Kebreab, E., Waghorn, G., Makkar, H.P., Adesogan, A.T., Yang, W., Lee, C., Gerber, P.J., Henderson, B., Tricarico, J.M., 2013. Special topics: mitigation of methane and nitrous oxide emissions from animal operations: I. A review of enteric methane mitigation options. J. Anim. Sci. 91:5045-5069.

Jouany, J.-P., 2001. A new look at yeast culture as probiotics for ruminants. Feed Mix 9:17-19.

Khattab, H.M., Gado, H.M., Kholif, A.E., Mansour, A.M., Kholif, A.M., 2011. The potential of feeding goats sun dried rumen contents with or without bacterial inoculums as replacement for berseem clover and the effects on milk production and animal health. Int. J. Dairy Sci. 6:267-277.

Kholif, A.E., Gouda, G.A., Morsy, T.A., Salem, A.Z.M., Lopez, S., Kholif, A.M., 2015. Moringa oleifera leaf meal as a protein source in lactating goat's diets: Feed intake, digestibility, ruminal fermentation, milk yield and composition, and its fatty acids profile. Small Ruminant Res. 129:129-137.

Kholif, A.E., Khattab, H.M., El-Shewy, A.A., Salem, A.Z.M., Kholif, A.M., El-Sayed, M.M., Gado, H.M., Mariezcurrena, M.D., 2014. Nutrient digestibility, ruminal fermentation activities, serum parameters and milk production and composition of lactating goats fed diets containing rice straw treated with Pleurotus ostreatus. Asian Austral. J. Anim. 27:357-364.

Kumar, D.S., Prasad, C.S., Prasad, R.M.V., 2013. Effect of yeast culture (Saccharomyces cerevisiae) on ruminal microbial population in buffalo bulls. Buffalo Bull. 32:116-119.

Kwiatkowski, S., Kwiatkowski, S.E., 2012. Yeast (Saccharomyces cerevisiae) glucan polysaccharides – occurrence, separation and application in food, feed and health industries. In: D.N. Karunaratne (ed.), The complex world of polysaccharides. InTech, Rijeka, Croatia, chapter 2. Available from: http://www.intechopen.com/books/the-complex-world-of-polysaccharides/yeast-saccharomyces-cerevisiae-glucan-polysaccharides-occurrence-separation-and-application-in-food-

Lille, S.H., Pringle, J.R., 1980. Reserve carbohydrate metabolism in Saccharomyces cerevisiae: response to nutrition limitation. J. Bacteriol. 143:1384-1394.

Mao, H.-L., Mao, H.-L., Wang, J.K., Liu, J.X., Yoon, I., 2013. Effects of Saccharomyces cerevisiae fermentation product on in vitro fermentation and microbial communities of low-quality forages and mixed diets. J. Anim. Sci. 91:3291-3298.

Martin, S.A., Nisbet, D.J., 1992. Effect of direct-fed microbials on rumen microbial fermentation. J. Dairy Sci. 75:1736-1744.

Martin, S.A., Nisbet, D.J., Dean, R.G., 1989. Influence of a commercial yeast supplement on in vitro ruminant fermentation. Nutr. Rep. Int. 40:395-403.

McGuffey, R.K., Richardson, L.F., Wilkinson, J.I.D., 2001. Ionophore for dairy cattle: current status and future outlook. J. Dairy Sci. 84:194-203.

Menke, K.H., Raab, L., Salewski, A., Steingass, H., Fritz, D., Schneider, W., 1979. The estimation of the digestibility and metabolizable energy content of ruminant feeding stuffs from the gas production when they are incubated with rumen liquor in vitro. J. Agr. Sci. 93:217-222.

Mosoni, P., Chaucheyras-Durand, F., Berat-Maillet, C., Forano, E., 2007. Quantification by real time PCR of cellulolitic bacteria in the rumen of sheep after supplementation of a forage diet with readily fermentable carbohydrates. Effect of a yeast additive. J. Appl. Microbiol. 103:2676-2685.

Mwenya, B., Santoso, B., Sar, C., Gamo, Y., Kobayashi, T., Arai, I., Takahashi, J., 2004. Effects of including 1-4 galacto-oligosaccharides lactic acid bacteria or yeast culture on methanogenesis as well as energy and nitrogen metabolism in sheep. Anim. Feed Sci. Tech. 115:313-326.

National Research Council, 2001. Nutrient requirements of dairy cattle, 7th rev. ed. National Academy Press, Washington, DC, USA.

Newbold, C.J., Wallace, R.J., McIntosh, F.M., 1996. Mode of action of the yeast Saccharomyces cerevisiae as a feed additive for ruminants. Brit. J. Nutr. 76:249-261.

Ørskov, E.R., McDonald, L., 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to the rate of passage. J. Agr. Sci. 92:499-503.

Pinloche, E., McEwan, N., Marden, J.-P., Bayourthe, C., Auclair, E., Newbold, C.J., 2013. The effects of a probiotic yeast on the bacterial diversity and population structure in the rumen of cattle. PLoS One 8:e67824.

Russell, J.B., Houlihan, A.J., 2003. Ionophore resistance of ruminal bacteria and its potential impact on human health. FEMS Microbiol. Rev. 27:65-74.

Salem, A.Z.M., Kholif, A.E., Elghandour, M.M.Y., Buendía, G., Mariezcurrena, M.D., Hernandez, S.R., Camacho, L.M., 2014a. Influence of oral administration of Salix babylonica extract on milk production and

composition in dairy cows. Ital. J. Anim. Sci. 13:10-14.

Salem, A.Z.M., Kholif, A.E., Olivares, M., Elghandour, M.M.Y., Mellado, M., Arece, J., 2014b. Influence of S. babylonica extract on feed intake, growth performance and diet in vitro gas production profile in young lambs. Trop. Anim. Health Pro. 46:213-219.

SAS, 2002. SAS user's guide: statistics, ver 9.0. SAS Inst. Inc., Cary, NC, USA.

Sedmak, J.J., 2006. Production of -glucans and mannans. US patent application Nov 23, 2006 US2006/0263415 A1. Available from: http://www.google.com/patents/US20060263415

Steel, R.G.D., Torrie, J.H., 1980. Principles and procedures of statistics. 2nd ed. McGraw-Hill Book Co., New York, NY, USA.

Sullivan, H.M., Martin, S.A., 1999. Effects of Saccharomyces cerevisiae culture on in vitro mixed ruminal microorganism fermentation. J. Dairy Sci. 82:2011-2016.

Tang, S.X., Tayo, G.O., Tan, Z.L., Sun, Z.H., Shen, L.X., Zhou, C.S., Xiao, W.J., Ren, G.P., Han, X.F., Shen, S.B., 2008. Effects of yeast culture and fibrolytic enzyme supplementation on in vitro fermentation characteristics of low-quality cereal straws. J. Anim. Sci. 86:1164-1172.

Theodorou, M.K., Williams, B.A., Dhanoa, M.S., McAllan, A.B., France, J., 1994. A simple gas production method using a pressure transducer to determine the fermentation kinetics of ruminant feeds. Anim. Feed Sci. Tech. 48:185-197.

Togtokhbayar, N., Cerrillo, S.M.A., Jigjidpurev, S., Shinekhuu, J., Urantulkhuur, D., Nergui, D., Elghandour, M.M.Y., Odongo, N.E., Kholif, A.E., 2015. Effect of exogenous xylanase on rumen in vitro gas production and degradability of wheat straw. Anim. Sci. J. 86:765-771.

Valdes, K.I., Salem, A.Z.M., Lopez, S., Alonso, M.U., Rivero, N., Elghandour, M.M.Y., Domínguez, I.A., Ronquillo, M.G., Kholif, A.E., 2015. Influence of exogenous enzymes in presence of Salix babylonica extract on digestibility, microbial protein synthesis and performance of lambs fed maize silage. J. Agr. Sci. 153:732-742.

Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fibre, neutral detergent fibre and non-starch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583-3597.

Wellens, A., Garafalo, C., Nguyen, H., Van Gerven, N., Slattegard, R., Hernalsteens, J.-P., Wyns, L., Oscarson, S., De Greve, H., Hultgren, S., Bouckaert, J., 2008. Intervening with urinary tract infections using anti-adhesives based on the crystal structure of the Fim Holigomannose-3 complex. PLoS One 3:1-13.

Wolin, M.J., Miller, T.L., 1988. Microbe-microbe interactions. In: P.N. Hobson and C.S. Stewart (eds.) The rumen microbial ecosystem. Springer, Heidelberg, Germany, pp 467-491.