British Journal of Applied Science & Technology
16(4): 1-8, 2016, Article no.BJAST.19166
ISSN: 2231-0843, NLM ID: 101664541

SCIENCEDOMAIN *international*
www.sciencedomain.org

Assessment of *in vitro* Gas and Methane Production of Diet Fortified with Yeast and *Lactobacilli spp.*

T. O. Ososanya[1*] and U. A. Inyang[2]

[1]Department of Animal Science, University of Ibadan, Ibadan, Nigeria.
[2]Department of Animal Science, University of Uyo, Uyo, Nigeria.

Authors' contributions

This work was carried out in collaboration between both authors. Author TOO designed the study, wrote the protocol and author UAI wrote the first draft of the manuscript and managed literature searches. Authors TOO and UAI carried out literature search and analyses of the study. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BJAST/2016/19166
Editor(s):
(1) Verlicchi Paola, Department of Engineering, University of Ferrara, Via Saragat 1, Ferrara, Italy.
Reviewers:
(1) Azhari Muhammad Syam, University of Malikussaleh, Indonesia.
(2) Roshanida A. Rahman, Universiti Teknologi Malaysia, Johor, Malaysia.
Complete Peer review History: http://sciencedomain.org/review-history/14900

Original Research Article

Received 28th May 2015
Accepted 17th July 2015
Published 4th June 2016

ABSTRACT

The effect of dietary fortification of two levels of bakers yeast and yeast plus *Lactobacilli* against negative control and positive control (antibiotic) was assessed on *in vitro* gas production (IVGP) kinetics and methane production at 24 hours incubation.
A concentrate diet was formulated and fortified with six levels consisting: control (D1); antibiotic (D2); 2.5 g bakers yeast (D3); 5.0 g bakers yeast (D4); 2.5 g yeast plus *Lactobacilli* (D5) and 5.0 g yeast plus *Lactobacilli* (D6) and mixed with *Panicum maximum* to serve as the substrate in a completely randomized design. The parameters tested were IVGP, organic matter digestibility (OMD), metabolizable energy (ME), short chain fatty acids (SCFA), methane gas, degradability, partitioning factor and microbial biomass. Higher ($P<0.05$) IVGP volumes, *in vitro* organic matter degradability, metabolizable energy (ME) and short chain fatty acid (SCFA) were recorded for diets D3 to D6 and D1 over D2, whereas the difference amongst D3 to D6 and D1 was not significant ($P>0.05$). Highest IVGP was recorded for D6 (16.33 ml) and the lowest (4.33 ml) in D2. Gas production from the soluble fraction (a), rate of constant of gas production (c) and time (t) were not significantly different ($p > 0.05$) while the gas production from the insoluble fraction (b), potential

**Corresponding author: E-mail: tososanya85@gmail.com, t.ososanya@mail.ui.edu.ng;*

extent of gas production (a + b) differed significantly ($p < 0.05$). For methane gas, D3 recorded the highest (5.00 ml / 200 mg DM) and D2 had the lowest (1.67 ml / 200 mg DM). All other fortifications were higher in methane gas than control. The present study demonstrated the potential of probiotics especially when mixed at 5 g level in improving degradation.

Keywords: Probiotics; in vitro gas production; methane; yeast; Lactobacilli.

1. INTRODUCTION

Livestock in the tropics subsist mainly on low quality roughages and this leads to low quality of output not forgetting the increase in population growth, dwindling grazing lands converted to cultivation of foods to meet the urgent human needs resulting in decreased land for fodder cultivation and forcing livestock to depend on alternate feed resources [1].

Methane production during anaerobic fermentation of nutrients in the rumen is an essential metabolic but nutritionally wasteful process which represents 2 to 15% of gross energy loss [2]. Reducing methane production is an important goal of ruminant nutritionists not only for reducing greenhouse gases and global warming but also for improving the efficiency of animal production [3].

Hence, several new technologies are being tried to improve their digestibility and utilization. One of such effort in recent years is supplementation of Probiotics to rations of livestock since it presents an attractive alternative to the use of chemical and hormonal promoters. They are known to improve the utilization of cellulosic materials, health, productivity and reproduction [1].

Studies had shown that the *in vitro* gas production was improved when supplemented with probiotics [4,1,5]. According to [6] the total gas production was higher in yeast supplemented groups under *in vitro* system. Methane production had been reported to increase by addition of yeast [7].

Mixed probiotics (yeast and lactobacilli) have been reported to record a higher *in vitro* gas production when compared with control and yeast supplemented diet. For methane gas, mixed probiotics supplemented diet was lower than control and sole *Lactobacilli* supplemented diet [4].

The objective of the present study was to further validate the effect of yeast and *Lactobacilli* in combination on *in vitro* gas production and methane.

2. MATERIALS AND METHODS

2.1 Experimental Site

Experiments were conducted at the Departments of Animal Science, University of Benin, Edo state and University of Ibadan, Oyo state.

2.2 Collection and Preparation of Experimental Diets

Concentrate diet was formulated as presented in Table 1 and mixed with *Panicum maximum* in a ratio of 60:40 (i.e. Roughage to concentrate). The *Panicum maximum* served as a basal diet while concentrate as a supplement. Bakers' yeast by Angel^{TM} was used as yeast source for D3 and D4 while yeast plus Lactobocilli was manufactured by Zoom^{TM} and used as fortification for D5 and D6 diets.

The diet were supplemented with no probiotic (D1 control), antibiotic (D2), bakers yeast (D3 and D4: 2.5 g and 5.0 g respectively) and yeast plus *Lactobacilli* (D5 and D6: 2.5 g and 5.0 g respectively).

2.3 *In vitro* Gas Production Procedure

In vitro gas production technique [8] was used to describe the extent of gas production from treatment diets. Rumen liquor was obtained with the help of a stomach tube, transferred into pre heated thermos flask, strained through a sieve cloth and flushed with CO_2. The buffer containing $NaHCO_3$ + Na_2HPO_4 + KCl + NaCl + $MgSO_4.7H_2O$ + $CaCl_2.2H_2O$ was used and kept in the incubator for warming prior to being mixed with rumen fluid (1:4) as inoculums, all under continuous flushing with streams of CO_2. About 200 mg of the substrate was measured and introduced into the syringe after removing the plunger. Incubation was carried out at 39±1ºC and volume of gas production was measured at 3 hourly intervals for 24 h. At post incubation period, 4 ml of NaOH (10M) was introduced to estimate methane production as reported by [9].

Table 1. Gross composition of concentrate feed mixture (%)

Ingredient (%)	D1	D2	D3	D4	D5	D6
Dried cassava peel	45	45	45	45	45	45
BDG	40.7	40.7	40.7	40.7	40.7	40.7
PKC	10	10	10	10	10	10
Limestone	2.5	2.5	2.5	2.5	2.5	2.5
Salt	1.5	1.5	1.5	1.5	1.5	1.5
Vitamin premix	0.30	0.30	0.30	0.30	0.30	0.30
Total	100	100	100	100	100	100
Yeast	-	-	2.5	5	-	-
Yeast+LAB	-	-	-	-	2.5	5
Antibiotic	-	+	-	-	-	-
Calculated CP	10.37	10.37	10.37	10.37	10.37	10.37

DCP – Dried Cassava Peals; BDG – Brewers Dried Grains; PKC – Palm Kernel Cake

The organic matter digestibility (%), metabolizable energy (ME, MJ kg/DM) and short chain fatty acid (SCFA, mmol/L) were calculated also degradability, partitioning factor and microbial biomass were estimated from the substrate truly degraded *in vitro* (mg).

2.4 Chemical and Statistical Analysis

Dried and ground samples of the feed were used for chemical analysis. Crude protein, crude fibre, ether extract and ash were determined according to methods of [10]. Neutral detergent fibre (NDF), Acid detergent fibre (ADF) and Acid detergent lignin (ADL) were determined using the methods of [11]. Data collected were statistically analyzed using [12] and the design was completely randomized.

3. RESULTS AND DISCUSSION

3.1 Chemical Composition of Feedstuff

The chemical composition of feed stuff/diet is presented in Table 2. The crude protein content ranged from 10.15% - 11.55%. The crude protein (CP) values obtained in this study was higher than the critical value of 7.70% or 70 g/Kg recommended for small ruminants [13] and also within range for the minimum protein requirement of 10 – 12% recommended by [14] for ruminants.

3.2 Incubation of Probiotic Fortified Diets

Table 3 shows the *in vitro* gas production of diets fortified with probiotics. There was no significant ($p > 0.05$) difference amongst the treatments in terms of gas production at the third (3^{rd}) h. However, from the 6^{th} to the 24^{th} h D6 recorded the highest gas volumes over the other treatments but similar to other treatments except for D2 from 6^{th} to 24^{th} h. The diet with antibiotics (D2) recorded the lowest gas production at all the hours. Gas volume increased with increasing hours for all the treatments signifying a high microbe action on the substrates; moreover a higher mixed probiotics (D6) recorded a higher gas volume when compared with control and D4. There was no significant ($p>0.05$) difference in gas production for control against test diets (D3 to D6) except in D2.

Table 2. Chemical composition of feedstuff (%)

Parameter	D1	D2	D3	D4	D5	D6	SEM
Dry matter	90.97^{cd}	91.60^{a}	91.41^{ab}	91.56^{ab}	91.26^{bc}	90.77^{d}	0.09
Crude protein	10.15^{b}	10.15^{b}	11.55^{a}	11.55^{a}	11.20^{a}	11.38^{a}	0.17
Crude fibre	11.00^{a}	11.50^{a}	11.50^{a}	11.50^{a}	11.50^{a}	11.50^{a}	0.26
Ash	8.00^{c}	10.00^{b}	10.00^{b}	8.50c	11.50^{a}	10.00^{b}	0.37
Ether extract	7.00^{a}	7.00^{a}	6.00^{b}	7.00^{a}	6.50^{ab}	6.50^{ab}	0.17
NDF	61.50^{a}	54.50^{e}	57.50^{d}	59.50^{b}	58.50^{c}	60.00^{b}	0.26
ADF	46.50^{b}	41.50^{e}	44.50^{c}	43.00^{d}	44.50^{c}	47.50^{a}	0.26
ADL	23.50^{a}	20.50^{c}	21.50^{b}	22.00^{b}	21.50^{b}	23.50^{a}	0.26
Hemicellulose	15.00^{b}	13.00^{d}	13.00^{d}	16.50^{a}	14.00^{c}	12.50^{d}	0.29
Cellulose	23.00^{a}	21.00^{b}	23.00^{a}	21.00^{b}	23.00^{a}	24.00^{a}	0.41

a,b,c, = Means on the same row bearing different superscripts differ ($p<0.05$) significantly
D1: -ve control; D2: +ve control (antibiotic); D3: Yeast 2.5 g/d; D4: Yeast 5.0 g/d; D5: LAB+Yeast 2.5 g/d and D6: LAB+Yeast 5.0 g/d

Table 3. *In vitro* gas production of diets fortified with probiotics for 24 h

Hours	D1	D2	D3	D4	D5	D6	SEM
3	2.33	0.67	1.00	1.00	1.33	1.67	0.64
6	6.00^{ab}	1.67^{c}	3.67^{bc}	5.33^{abc}	5.00^{abc}	8.33^{a}	1.21
9	9.00^{a}	1.67^{b}	6.67^{a}	8.33^{a}	6.67^{a}	8.33^{a}	1.46
12	11.33^{a}	1.67^{b}	8.00^{a}	10.67^{a}	8.67^{a}	10.67^{a}	1.81
15	12.67^{a}	1.67^{b}	7.67^{a}	11.33^{a}	8.67^{a}	12.00^{a}	1.82
18	12.00^{a}	2.00^{b}	7.67^{a}	13.00^{a}	9.00^{a}	11.67^{a}	1.81
21	12.33^{a}	2.67^{b}	9.33^{a}	14.33^{a}	11.00^{a}	14.67^{a}	1.61
24	12.00^{ab}	3.67^{c}	9.00^{b}	14.33^{a}	11.00^{ab}	14.67^{a}	1.60

A,b,c means with the same superscript among row are not significantly different (P>0.05)
D1: -ve control; D2: +ve control (antibiotic); D3: Yeast 2.5 g/d; D4: Yeast 5.0 g/d; D5: LAB+Yeast 2.5 g/d and D6: LAB+Yeast 5.0 g/d

Table 4. *In vitro* fermentation characteristic of probiotics fortified diets

Treatment	a	b	a+b	c	t	Y
D1	2.33	12.00^{ab}	14.33^{ab}	0.14	8.00	8.33^{a}
D2	0.67	3.67^{c}	4.33^{c}	0.08	11.00	2.00^{b}
D3	1.00	9.00^{b}	10.00^{b}	0.15	8.00	6.67^{a}
D4	1.00	14.33^{a}	15.33^{ab}	0.08	7.00	6.78^{a}
D5	1.33	11.00^{ab}	12.33^{ab}	0.11	11.00	7.00^{a}
D6	1.67	14.67^{a}	16.33^{a}	0.13	8.00	10.00^{a}
SEM	0.63	1.60	1.83	0.05	3.24	1.29

A,b,c means with the same superscript among column are not significantly different (P>0.05)
D1: -ve control; D2: +ve control (Antibiotic); D3: Yeast 2.5 g/d; D4: Yeast 5.0 g/d; D5: LAB+Yeast 2.5 g/d and D6: LAB+Yeast 5.0 g/d; Gas production from soluble fraction (a), insoluble fraction (b); rate of constant of gas production (c) and time (t)

However, as also observed in Table 4 showing *in vitro* fermentation characteristics of probiotics fortified diets, D6 recorded the highest gas volume (14.67 ml) numerically when compared with control D1 (12.00 ml) but significantly (P<0.05) higher than that of D2 (3.67 ml). The result obtained have is in agreement with [15], who reported that total gas increased with increase yeast supplementation. However, only at 5 g level of D4 and D6 that the total gas surpassed control (D1), therefore, indicating a better nutrient availability for rumen microorganisms [16]. Yeast plus *Lactobacilli* supplemented in roughage to concentrate diet (60:40) elicited a higher total gas volume when compared with yeast alone and *Lactobacilli* supplementations as reported by [4]. The high gas production seen in D4 and D6 might be due to the stimulatory effect of a higher dosage of the probiotics on microorganisms that improved degradability and in turn the gas profile. Fig. 1 shows the graphical illustration of *in vitro* gas production of diets fortified with probiotics at 24 h incubation.

The same pattern of total gas production is observed for estimated parameters (i.e. ME, OMD and SCFA) in Table 5. Highest inclusion yeast alone and yeast plus *Lactobacilli* elicited higher values over control (D1) and D2 recorded the least in all the parameters. The reason for these can not be farfetched and can be attributed to the effect of antibiotics in microorganisms (i.e. reducing the amount of methanogens) [17] thereby affecting degradation rate and in turn gas volumes and estimated parameters.

Table 5. Estimated parameters of feedstuff fortified with probiotics

Treatment	ME (MJ/Kg DM)	OMD (%)	SCFA (mmol/L)
D1	4.37^{ab}	35.32^{ab}	0.23^{ab}
D2	3.29^{c}	29.22^{c}	0.06^{c}
D3	4.06^{b}	34.59^{b}	0.16^{b}
D4	4.76^{a}	38.35^{ab}	0.28^{a}
D5	4.30^{ab}	37.20^{ab}	0.20^{ab}
D6	4.19^{a}	39.55^{a}	0.29^{a}
SEM	0.21	1.43	0.04

A,b,c means with the same superscript among column are not significantly different (P>0.05)
D1: -ve control; D2: +ve control (Antibiotic); D3: Yeast 2.5 g/d; D4: Yeast 5.0 g/d; D5: LAB+Yeast 2.5 g/d and D6: LAB+Yeast 5.0 g/d

3.3 Effect of Fortification on Methane Gas

Results of the present study indicated (Fig. 2) that methane production was decreased drastically (1.67 ml) in D2 while D3 recorded the highest volume (5.00 ml). Methane gas reduced by 45.50% in D2 while probiotic fortified (D3-D6) increased by 36.24%, 27.25%, 8.99% and 18.26% respectively when compared with control value. The reduction seen in D2 was due to reduction in methanogenesis as a result of antibiotic effect on protozoan population which may have led to a reduced availability of hydrogen ions for methane production by methanogens [18]. Yeast plus *Lactobacilli* at 2.5 g was effective in reducing methane and this is in contrast with the findings of [4] who reported that probiotics (yeast vs. yeast *Lactobacilli*) did not reduce methane production.

The result from the present study agreed with that of [7] who reported that methane production was increased by the addition of yeast. This was observed in D3 and D4 which were higher than those of D5 and D6 (5 and 4.67 ml vs. 4 and 4.34 ml respectively).

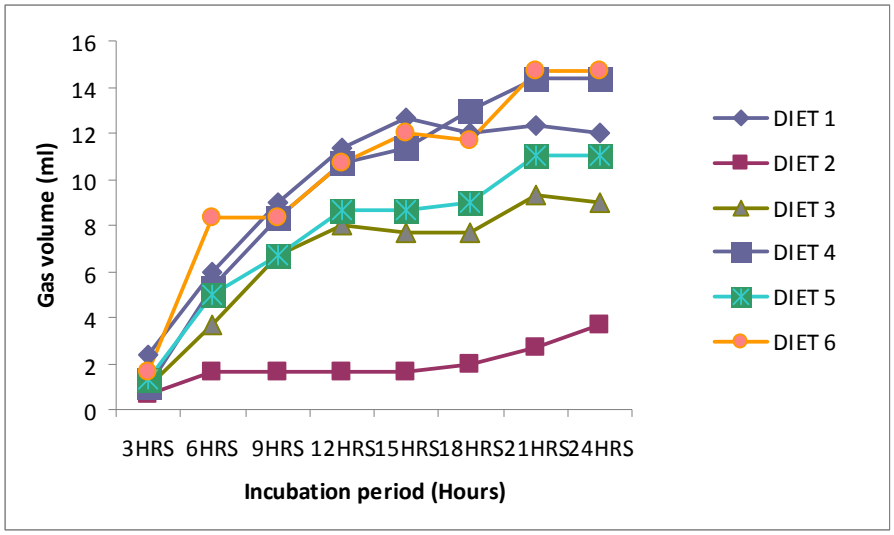

Fig. 1. *In vitro* gas production of feedstuff at 24 h

D1: -ve control; D2: +ve control (antibiotic); D3: Yeast 2.5 g/d; D4: Yeast 5.0 g/d; D5: LAB+Yeast 2.5 g/d and D6: LAB+Yeast 5.0 g/d

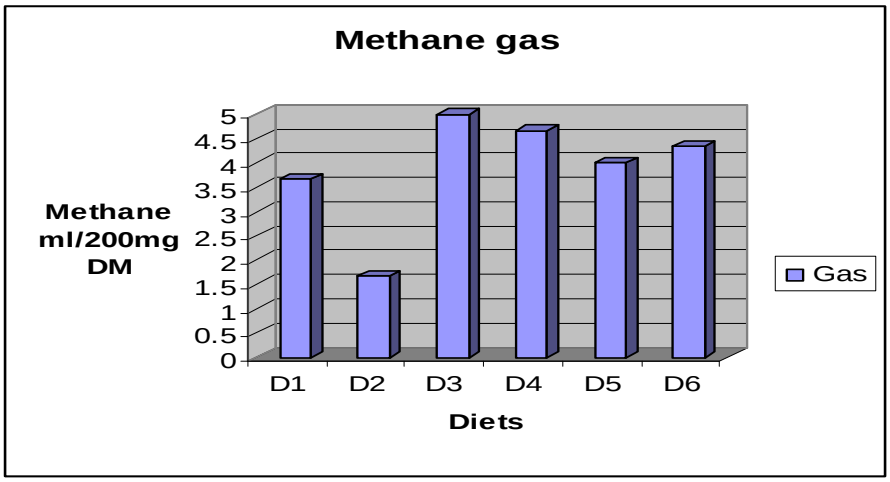

Fig. 2. Methane gas at 24 h incubation

D1: -ve control; D2: +ve control (antibiotic); D3: Yeast 2.5 g/d; D4: Yeast 5.0 g/d; D5: LAB+Yeast 2.5 g/d and D6: LAB+Yeast 5.0 g/d

3.4 Degradability, PF and MBM

Fig. 3 shows the percentage (%) of degraded and undegraded substrate after 24 h incubation. As observed from the figure, D6 was degraded the most (172.00 mg) while D2 recorded the least (124.67 mg) significantly ($p< 0.05$) when compared with D6. In terms of undegraded substrate D2 had the highest (75.33 mg) while D6 recorded the least (28.00 mg). Since probiotics have been shown to improve gas production and also acts as an activator of ruminal fermentative processes [4,19] it therefore invariably would mean that degradation will also increase and indicate a better nutrient availability for rumen microorganisms [16].

Table 6 presents the effects of probiotics fortified diets on degradability, partitioning factor (PF), microbial biomass (MBM) and nitrogen degradability. The rate of degradability per hour of incubation (Rdegr/h) was highest ($p < 0.05$) for D6 (7.17 mg/h) and least in the antibiotic treated diet (D2) at 5.19 mg/hr. The antibiotic might have affected the population of certain organisms thereby affecting the rate of degradation by slowing it down when compared with others. However, in a twist of fate the PF was highest ($p < 0.05$) for D2 at 9.06 mg/ml and least value was recorded for D4 (4.65 mg/ml). The antibiotic treated diet was significantly ($p < 0.05$) different from the other diets. The PF is an index of the distribution of truly degraded substrate between microbial biomass and fermentation waste products. When less gas is produced per unit weight of substrate truly degraded, proportionately more substrate is converted into microbial biomass, which means that, a higher PF would reflect higher conversion of truly degraded substrate into microbial biomass and vice versa [20,21]. In this study, diets having higher IVGP and degradability (D6<D4<D1<D3) were recording lower MBM and PF values, indicating an inverse relationship between IVGP and MBM. Moreover, the result obtained in this study is in agreement with [20,21,22].

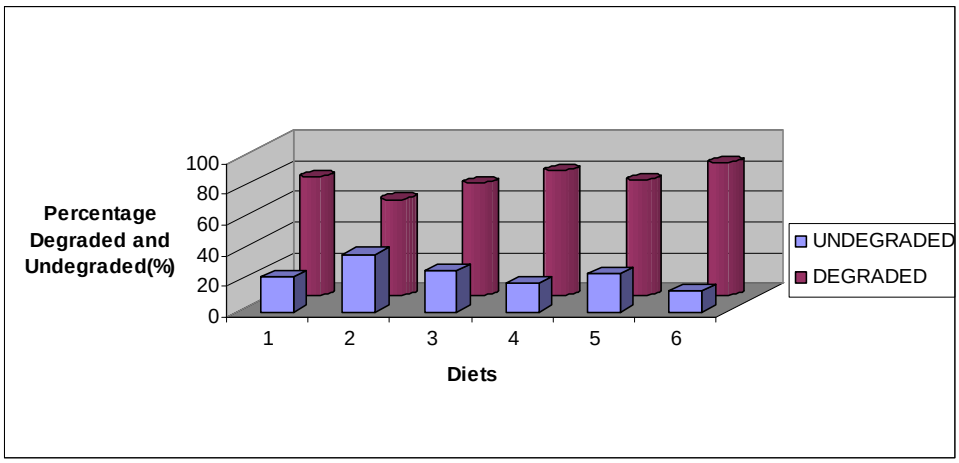

Fig. 3. Percentage degraded and undegraded substrates fortified with yeast and LAB
D1/T1: -ve control; D2/T2: +ve control (antibiotic); D3/T3: Yeast 2.5 g/d; D4/T4: Yeast 5.0 g/d; D5/T5: LAB+Yeast 2.5 g/d and D6/T6: LAB+Yeast 5.0 g/d

Table 6. Effect of yeast and LAB fortification on degradability, PF and MBM

Treatment	Degr	Undegr	Rdegr/h	PF	MBM	Ndegr
D1	154.00^{ab}	46.00^{ab}	6.42^{ab}	5.08^{b}	42.17	5.21^{ab}
D2	124.67^{b}	75.33^{a}	5.19^{b}	9.06^{a}	46.58	4.24^{b}
D3	145.33^{ab}	54.67^{ab}	6.06^{ab}	6.01^{b}	44.92	5.60^{ab}
D4	162.00^{ab}	38.00^{ab}	6.75^{ab}	4.65^{b}	41.25	6.22a
D5	150.67^{ab}	49.33^{ab}	6.28^{ab}	5.49^{b}	43.08	5.63^{ab}
D6	172.00^{a}	28.00^{b}	7.17^{a}	4.91^{b}	45.50	6.53^{a}
SEM	6.72	6.72	0.28	0.95	7.11	0.50

A,b,c means with the same superscript among column are not significantly different (P>0.05)
D1: -ve control; D2: +ve control (antibiotic); D3: Yeast 2.5 g/d; D4: Yeast 5.0 g/d; D5: LAB+Yeast 2.5 g/d and D6: LAB+Yeast 5.0 g/d; Degr: Degraded; Undegr: Undegraded; Rdegr/h: Rate of degradation per hr; PF: Partitioning factor; MBM: Microbial biomass; Ndegr: Nitrogen degradation

4. CONCLUSION

The results of the *in vitro* gas production study indicated that the yeast plus *Lactobacilli* fortification at 5g/d had a significant impact on gas production and estimated parameters when compared with control and other probiotic fortified diets. Also it further validates the remarkable potential of probiotics in mixed culture (i.e. yeast and *Lactobacilli*) over single yeast in small ruminant diets/rations through its stimulatory and buffering effect in the rumen that brings about higher gas production and degradation.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Harikrishna Ch, Mahender M, Ramana RY, Gnana PM, Sudhakar K, Pavani M. Evaluation of *in vitro* gas production and nutrient digestibility of complete diets supplemented with different levels of thermotolerant yeast in Nellore rams. Vet. World. 2012;5(8):477-485.
2. Moss AR, Jouany J, Newbold J. Methane production by ruminants: Its contribution to global warming. Animal Research. 2000; 49(3):231–253.
3. Sirohi SK, Goel N, Singh N. Influence of *Albizia lebbeck* saponin and its functions on *in vitro* gas production kinetics, rumen methanogenesis, and rumen fermentation characteristics. ISRN Veterinary Science; 2014. Article ID 498218: 1-10.
4. Kumar M, Dutta TK, Singh G. Effect of *Saccharomyces cerevisae* and *Lactobacilli* supplementation on methane production of diets with varying roughage and concentrate ratio. Indian Res. J. Genet. and Biotech. 2014;6(1):330-334.
5. Takahashi J, Miyagawa T, Kojima Y, Umetsu K. Effects of Yucca shidigera extract, probiotics, monensin and L-cysteine on rumen methanogenesis. Asian-Aust. J. Anim. Sci. 2000;13(A):499-501.
6. Malik R, Singh R. Effect of yeast and fungal culture on *in vitro* ruminal fermentation. Indian J. Anim. Nutri. 2009;26(1):40-45.
7. Ando S, Khan RI, Takahashi J. Manipulation of rumen fermentation by yeast: the effects of dried beer yeast on the *in vitro* degradability of forages and methane production. Asian –Australian Journal of Animal Sci. 2004;17:68-72.
8. Menke KH, Steingass H. Estimation of energetic feed value obtained from chemical analysis and *in vitro* gas production using rumen fluid. Anim. Res. Dev. 1988;28:7-55.
9. Fievez V, Babayemi OJ, Demeyer D. Estimation of direct and indirect gas production in syringes: A tool to estimate short chain fatty acid production requiring animal laboratory facilities. Anim. Feed Sci. Technol. 2005;123:197-210.
10. A. O. A. C. Association of Analytical chemist. Official methods of analysis. 15th edition. Washington DC. 1990;15:69-88.
11. Van Soest PJ, Robertson JB, Lewis BA. Methods for dietary fiber, neutral detergent fiber and non starch polysaccharides in relation to animal nutrition. J. Dairy Sci. 1991;74:3583-3597.
12. S.A.S. Statistical analysis system. SAS Institute Inc. Cary. North Carolina, USA. 1999;8.
13. National Research Council (NRC). Nutrient requirement of goats: Angora, dairy and meat in temperate and tropical countries. 1st edn. National Academy Press, Washington DC. 1981;1.
14. A.R.C. Agricultural Research Council. The nutrient requirements of farm animals. No. 2, Ruminants: Tech. Rev. and Summaries. ARC, London. 1985;2.
15. Wolin MJ, Miller TL. Microbe interactions In: Rumen microbial ecosystem. P. N. Hobson, ed. Elsevier Science, Essex, U.K. 1988;343.
16. Ahmed GM, Abdel NMAFE. Chemical composition and *in vitro* gas production characteristics of six fodder trees, leaves and seeds. Res. J. Agric. Biol. Sci. 2007; 3(6):983-986.
17. Church DC, editor. The ruminant animal digestive physiology and nutrition. Prentice Hall Inc., USA. 1988;139.
18. Patra AK, Saxena J. Dietary phytochemicals as rumen modifiers: A review of the effects on microbial populations. Antonie van Leeuwenhoek. 2009;96(4):363-375.
19. Galindo J, Torres V, Rodriguez Z, Alvarez E, Aldana AI, Rodriguez R, Garcia R, Boucourt R, Delgado D, Martin E, Moreira O, Noda A, Nunez O, Gonzalez M, Herrera F, Cairo J, Rodriguez F). Avances en el studio de las levaduras como activadoras

de la fermentacion ruminal en bovinos que consumen dietas fibrosas. Ciencia en la Frontera. Rev de Cien. Y Tec. De la UACJ. 2008; VI. Numero especial: 93. Spanish.

20. Thirumalesh T, Krishnamoorthy U. Effect of feeding diets differing in partitioning factor (PF) on intake, digestibility and nitrogen metabolism in ram lambs. Anim. Nutr. Feed Technol. 2009;9:11-20.
21. Blummel M, Karsli A, Russel JR. Influence of diet on growth yields of rumen microorganisms in vitro and in vivo: influence on growth yield of variable carbon fluxes to fermentation products. Br. J. Nutr. 2003;90:1-11.
22. Blummel M, Steingass H, Becker K. The relationship between *in vitro* gas production, *in vitro* microbial biomass yield and N. incorporation and its implications for the prediction of voluntary feed intake of roughages. Br. J. Nutr. 1997;77:911-921.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/14900