ASSESSMENT OF *IN VITRO* GAS AND METHANE PRODUCTION OF DIET FORTIFIED WITH YEAST AND *LACTOBACILLI*

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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 24hours incubation. A concentrate diet was formulated and fortified with six levels consisting: (D1); antibiotic (D2); 2.5g bakers yeast(D3); 5.0g bakers yeast(D4); 2.5g yeast plus Lactobacilli (D5) and 5.0g yeast plus Lactobacilli (D6) and mixed with Panicum maximum. Higher (P0.05) IVGP volumes, invitro 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 (16.33ml) and the lowest (4.33ml) 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 extent of gas production (a + b) differed significantly (p < 0.05). For methane gas, D3 recorded the highest (5.00ml/200mg DM) and D2 had the lowest (1.67ml/200mg Dm). All other fortifications were higher in methane gas than control. The present study demonstrated the potential of probiotics especially when mixed at 5g level in improving degradation.

Keywords: Probiotics, *in vitro* gas production, methane, Yeast, Lactobacilli.

Introduction

Livestock in the tropics subsist mainly on low quality roughages and this leads to low quality of output thereby forcing livestock to depend on alternate feed resources (Harikrishna *et al.*, 2012). 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 (Moss *et al.*, 2000). 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 (Sirohi *et al.*, 2014). Hence, several new technologies are being tried to improve their digestibility and utilization. One of such effort is supplementation of Probiotics to rations of livestock since it presents an attractive alternative to the use of antibiotics. Studies had shown that the *in vitro* gas production was improved when supplemented with probiotics (Kumar *et al.*, 2014; Takahashi *et al.*, 2000). According to Malik and Singh (2009) the total gas production was higher in yeast supplemented groups under *in vitro* system. Methane production has been reported to increase by addition of yeast (Ando *et al.*, 2004) while Kumar *et al.* (2014) reported that mixed probiotics supplemented diet recorded lower methane than control and sole *Lactobacilli* supplemented diet. The objective of the present study was to further validate the effect of yeast and Lactobacilli either on *in vitro* gas production and methane.

Materials and Methods

Experimental site and diets

Experiments were conducted at the Departments of Animal Science, University of Benin, Edo state and University of Ibadan, Oyo state. Concentrate diet was formulated as presented in Table 1 and mixed with *Panicummaximum* in a ratio of 60:40 (i.e. Roughage to concentrate). Bakers' yeast by Angel™ was used as yeast source for D3 and D4 while yeast plus *lactobocilli* was manufactured by Zoom™ 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: 2.5g and 5.0g respectively) and yeast plus lactobacilli (D5 and D6: 2.5g and 5.0g respectively).

In vitro gas production

In vitro gas production technique (Menke and Steingass, 1988) 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. About 200 mg of the silage (substrate) was measured and introduced into the syringe after removing the plunger. Incubation was carried out at 39±1°C and volume of gas production was measured at 3 hourly intervals for 24hours. At post incubation period, 4ml of NaOH (10m) was introduced to estimate methane production as reported by (Fievez *et al.*, 2005). The organic matter digestibility (%), metabolizable energy (ME, MJ kg/DM) and short chain fatty acid (SCFA, mmol/L) were calculated.

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 (AOAC, 1990). Neutral detergent fibre (NDF), Acid detergent fibre (ADF) and Acid detergent lignin (ADL) were determined using the methods of (Van Soest *et al.*, 1991). Data collected were statistically analyzed using (SAS, 1999) and the design was completely randomized.

Results and Discussion:

The chemical composition of feed stuff/diet is presented in Table 2. The crude protein content ranged from 10.15% - 11.55% and was higher than the critical value of 7.70% or 70 g/Kg recommended for small ruminants (NRC, 1981).

Table 3 shows the *in vitro* gas production fermentation characteristics of diets fortified with probiotics. The insoluble but potentially degradable fraction (b) and potential extent of gas production (a+b) was influenced by the fortification of probiotics by increasing the gas production with mixed probiotic being the highest and in agreement with findings of Doto and Liu (2011).

The reason for this increase can be adduced to promotion of the growth of rumen bacteria and optimization of rumen function by combinations of yeast and bacteria probiotic that exerted synergestic effects (Chaucheyras *et al.*, 2008). Figure 1 shows the graphical illustration of *in vitro* gas production of diets fortified with probiotics at 24 hour incubation. The same pattern of gas fermentation characteristics is observed for estimated parameters (i.e. ME, OMD and SCFA) in Table 4. The reason for low values of D2 can be attributed to the effect of antibiotics in microorganisms (i.e. reducing the amount of methanogens) (Church, 1988).

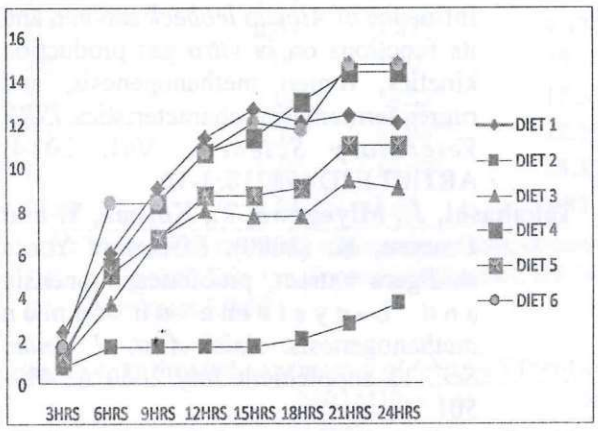

Figure 1: In vitro gas production at 24 hours
D1/T1: -ve Control; D2/T2: +ve Control (Antibiotic); D3/T3: Yeast 2.5g/d; D4/T4: Yeast 5.0g/d; D5/T5: LAB+Yeast 2.5g/d and D6/T6: LAB+Yeast 5.0g/d

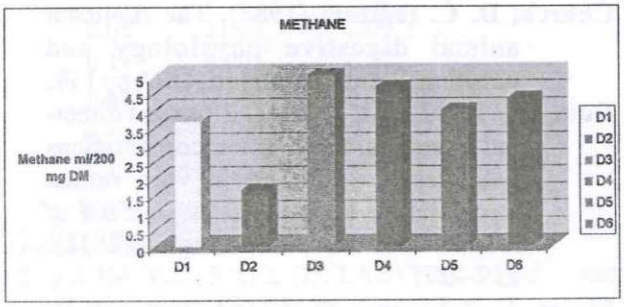

Figure 2:Figure 2: methane gas at 24 hours incubation

Results of the present study indicated (Figure 2) that methane production was decreased drastically (1.67ml) in D2 while D3 recorded the highest volume (5.00ml). 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 (Patra and Saxena, 2009). Yeast plus lactobacilli at 2.5g was effective in reducing methane and this is in contrast with the findings of (Kumar *et al.*, 2014) who reported that probiotics (yeast vs. yeast lactobacilli) did not reduce methane production. The result from the present

study agreed with that of (Ando *et al.*, 2004) who reported that methane production was increased by the addition of yeast.

Conclusion

The results of the *in vitro* gas production study indicated that the yeast plus lactobacilli fortification at 5g can be incorporated in small ruminant, rations without any adverse effects. Also the study further validates the potential of probiotics in small ruminant diets/rations through its stimulatory and buffering effect in the rumen that brings about higher gas production.

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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

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

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 2.5g/d; D4: Yeast 5.0g/d; D5: LAB+Yeast 2.5g/d and D6: LAB+Yeast 5.0g/d

Table 3: *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 2.5g/d; D4: Yeast 5.0g/d; D5: LAB+Yeast 2.5g/d and D6: LAB+Yeast 5.0g/d

Table 4: Estimated parameters of feedstuff fortified with probiotics

	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	4.79^{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 ($P>0.05$)