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IN VIVO ENTERIC METHANE MITIGATION USING *Saccharomyces cerevisiae* IN WEST AFRICAN DWARF SHEEP FED *Panicum maximum* AND *Centrosema pubescens*

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ABSTRACT

Decreasing enteric methane production in ruminants without altering their overall productivity is one of the strategic means of mitigating the global greenhouse gas emission from ruminants and also improving feed conversion efficiency. In this study, we investigated the effect of bioactive yeast (Saccharomyces cerevisiae) on enteric methane production from West African Dwarf Sheep (West African Dwarf Sheep) fed Panicum maximum and Centrosema pubescens. Three graded dose levels (0.4, 0.6 and 0.8 grams per kilogram body weight) of SC were orally administered to three groups (A, B and C) of five West African Dwarf Sheep respectively. Another group D of same animal number served as the control. In vivo methane production was estimated using appropriate prediction equation. Panicum maximum and Centrosema pubescens were fed to all the groups. The results of the study showed that compared to the untreated control, supplementation of both P. maximum and C. pubescens diet groups with SC significantly ($p < 0.05$) reduced methane production in a dose dependent manner; increasing doses resulting in decreasing levels of methane production and vice versa.. The reduction in methane emission for group C that received SC at 0.8 g/kg body weight was 11.38% and 15.85% respectively for P. maximum and Centrosema pubescens diet groups. The overall productivity of the West African Dwarf Sheep however, was not negatively affected by the bioactive yeast. These observations suggest that regulated dietary inclusion of bioactive yeast can be used to bioengineer the rumen towards mitigation of enteric methane production and efficient feed conversion.

***Keywords*:** *Saccharomyces cerevisiae*, Forages, Methane mitigation, Sheep.

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INTRODUCTION

Climate change is a subject of global environmental concern. Increased anthropogenic greenhouse gas (GHG) emissions have increased the global temperature the last 100 to 200 years. Global surface temperatures are predicted to increase by 1 - 6°C during the twenty-first century, primarily due to increased levels of GHGs principally carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) in the atmosphere [1,2,3].

Methane is a very important greenhouse gas since it has been reported to have an effect that is 21-times greater than that of carbon dioxide in terms of global warming [4]. Enteric methane from livestock constitute 65% of total agricultural GHG emissions, which renders ruminant livestock industries highly vulnerable to carbon trading systems in which a financial penalty is associated with GHG emission [5].
It has been variously predicted that world temperatures will rise about 0.5 – 2.5°C by 2030 [6,7] with a concomitant rise in global mean sea level of about 17 - 26 cm due mainly to thermal expansion of the oceans and increased melting of ice in the Arctic and Antarctic areas. Chaotic weather changes may result in droughts or floods and eventually massive erosions. The rise in temperature will also alter precipitation patterns and trigger extreme weather conditions which can threaten fresh water sources, change delicate ecosystems. It will further disrupt the farming, fishing, forestry and many other industries that rely on the weather and natural ecosystems resulting in decreased crop yields and available arable land and subsequently starvation and malnutrition. This rise in temperature will alter the range of diseases that threaten animal and/or human health while lowering the immune system. In general, climate change will affect livestock productivity directly by influencing the balance between heat dissipation and heat production and indirectly through its effects on the availability of feed and fodder [8,9].

On the basis of the foregoing, consideration of the contributions of ruminants to global warming should be a serious matter that requires urgent discussion and practical investigation. This specifically has to do with the quantity of methane (CH_4) emitted by ruminants and its mitigation strategies. On the average, a sheep produces about 30 litres of CH_4 each day and a dairy cow up to 200 litres per day [10]. Enteric CH_4 is produced under anaerobic conditions in the rumen, by methanogenic Archaea, utilising carbon dioxide (CO_2) and Hydrogen (H_2) to form CH_4; thus reducing metabolic H_2 produced during microbial metabolism [11]. If H_2 accumulates, re-oxidation of nicotinamide adenine dinucleotide (NADH) will be inhibited, thus inhibiting microbial growth, forage degradability and the associated production of volatile fatty acids (acetate, propionate and butyrate). Therefore, any mitigation strategy aimed at reducing methanogen populations must also include an alternative pathway for H_2 removal from the rumen. Typically, about 6 to 10% of the total gross energy consumed by a dairy cow is converted to CH_4 and released via eructation; thus, reducing enteric CH_4 production may also lead to some production benefits [12].

The various methods available for mitigation of methane emission from ruminants include the direct inhibition of methanogenesis by halogenated methane analogues and related compounds such as chloroform, bromochloromethane, α-cyclodextrin, amichloral, 2-bromoethanesulfonic acid, etc, but most of them are either toxic, only effective in vitro or their effects are transient [13,14,15]. Other mitigation strategies for methane include the elimination of protozoa [16,17]; the use of ionophoric antibiotic like monensin [18]; the use of propionate enhancer like dicarboxylic organic acids such as malate and fumarate [19,20] and diet type [9].

Available reports suggested that the addition of *Saccharomyces cerevisiae* (SC) to an in vitro system initially reduced methane production by 10% although this was not sustained [21]. Probiotics work efficiently in diets with low crude protein and high energy [22,23]. The mode of action of bioactive yeast in rumen bioengineering is thought to be mediated through microbial stimulation, oxygen sequestration and pH modulation [24].The use of probiotics in animal nutrition and hence research involving yeast is new in the tropics and has been directed mainly on poultry [25]. In order to evaluate the possible impact of yeast on enteric methane production by ruminants, this study was designed to determine the effect of

supplementation of bioactive yeast (*Saccharomyces cerevisiae*) on the mitigation of enteric methane production in WAD sheep fed *Panicum maximum* and *Centrosema pubescens*.

MATERIALS AND METHOD

Experimental Animals

Twenty adult female WAD sheep purchased from local markets in Nsukka and Udenu Local Government Areas of Enugu State, Nigeria were used for this study. After purchase, they were quarantined for 14 days and acclimatized for another 21 days at the Experimental Animal Unit of the Faculty of Veterinary Medicine Teaching and Research Farm, University of Nigeria, Nsukka. They had an average body weight of 12.78 ±1.95 kg after acclimatization. They were treated for both ecto- and endo-parasites using ivermectin at 200 µg/kg body weight subcutaneously (ivomec®, Argenta Manufacturing Limited, Auckland, New Zealand) and levamisole hydrochloride at 7.5 mg/kg body weight (bolus). They were also vaccinated against peste des petit ruminants using the tissue culture vaccine (National Veterinary Research Institute, Vom, Nigeria). The sheep were identified using ear tag plates and housed individually in wooden well- ventilated metabolic crates in fly-proof pens.

Feeding /Management

At the time of quarantine, the animals were fed *ad libitum* with freshly harvested *P. maximum* (PM) and *C. pubescens* (CP) and water. The animals were restricted to PM during the period of acclimatization. At each feeding, the forage samples were weighed using a scale and tied on a pole in the interior of the cages to prevent faecal contamination. Left over feed sample was collected and weighed the following day to determine the actual feed intake.

Forage samples

PM (guinea grass) and CP (a legume) were harvested and identified using a specimen sample at the herbarium of the Department of Botany, University of Nigeria, Nsukka. Fresh forage samples were also sent to the laboratory to determine their chemical composition.

Experimental Treatment

The twenty sheep were divided into four groups (A, B, C and D) of five sheep each. The experiment was divided into two protocols beginning with *P. maximum*. Feeding was restricted to *P. maximum* for 28 days. Supplementation with 0.4, 0.6 and 0.8 grams of SC/kg body weight were administered to animals in groups A, B and C respectively every day during the treatment period. Group D served as unsupplemented control. Fresh faecal and blood samples were collected from each animal in a group before and at the end of the treatment period for that group. Faecal samples were analyzed to determine the proximate composition of soluble residue, hemicelluloses and cellulose [26]. Haematological parameters were determined according to Schalm *et al*. [27]. Similar protocol groups and procedures were repeated with *C. pubescens* after an adjustment (cross over) period of 28 days.

In both protocols, methane production was determined by fitting soluble residue, hemicelluloses and cellulose composition of faeces into the prediction equation of Moe and Tyrrell [28] as follow:

$$Methane\ (CH4) = 3.406 + 0.51\ (soluble\ residue) + 1.736\ (hemicellulose) + 2.648\ (cellulose)$$

Where: CH_4 is in mega joules/day and soluble residue, hemicellulose, and cellulose in kg feed/day. The hemicellulose component of the faeces was determined as the difference between the neutral detergent fibre (NDF) and the acid detergent fibre (ADF).

DATA ANALYSIS

The data collected for each parameter studied were analyzed using the SPSS version 16.0 statistical package. Means were taken to be statistically significant at $p < 0.05$ whereas separation of the means was done using the Duncan's New Multiple Range Test [29].

RESULTS

The proximate analyses of the various diets are presented in Table 1. In Table 2, the soluble residue in the PM diet in group A (9.41 ± 0.02 %) was similar ($p > 0.05$) to group C (9.39 ± 0.03 %) but significantly ($p < 0.05$) lower than those of groups B (9.70 ± 0.02 %) and D (9.63 ± 0.01 %) in the PM diet. In the CP diet group, soluble residue values were generally higher in the treatment groups than the control and significantly ($p < 0.05$) so between groups B and D.

Table 1: Proximate analysis of the various diets

Chemical constituents (%)	**Diet groups**	
	Panicum maximum	***Centrosema pubescens***
Dry matter (DM)	46.35	47.12
Organic matter (OM)	28.65	27.03
Crude protein	14.53	18.95
Crude fibre	58.00	47.77
Fats	2.38	1.60
Ash	6.63	6.37
Calcium	4.0	4.12
Potassium	0.5	0.89
Phosphorus	4.77	7.22
Sodium	0.53	0.58
Neutral detergent fibre (NDF)	75.28	68.50
Acid detergent fibre (ADF)	43.45	37.23
Hemicellulose	50.20	39.04
Cellulose	42.34	37.11

Methane production was significantly ($p < 0.05$) higher in the control group than any of the treatment groups irrespective of the diet fed (Table 2). Among the diet groups, the addition of SC influenced methane production in a dose-dependent manner; higher doses of SC resulting in lower levels of methane production and vice versa irrespective of the diet fed. Methane production was however generally lower in all treatment groups as well as the control when the animals were fed with CP than PM.

The hemicelluloses, cellulose, ADF and NDF followed a similar trend as methane production with higher values being recorded in the control than any of the treatment groups and group A which received the lowest doses of SC having the highest methane emission among the treatment groups in both CP and PM diet groups.

DISCUSSION

This study has provided information on the mitigation effect of a probiotic, *Saccharomyces cerevisiae* (bioactive yeast) on methane production in West African Dwarf sheep (WADS). The study showed that varied doses of bioactive yeast affected methane production in a dose dependent manner. The varied inclusion levels of SC reduced methane production significantly, when compared with the control group. The reduction might have been mediated through the stimulation of acetogenic microbes in the rumen which consume hydrogen (H_2) to form acetate; thus potentially reducing methane production [30,31]. It is

also possible that the reduction might be through the modulation of rumen pH by stimulating entodiniomorphid protozoa that engulf starch particles thereby preventing their fermentation to lactate; or through competition with amylolytic bacteria for starch [32,33], and fermentation of starch (at a slower rate) to volatile fatty acids with lower dissociation and acidogenic potential than lactate [24]. The results agree with the findings of Mutsvangwa *et al*. [21] who reported a 10% reduction in methane production in an in vitro system when SC was added. Similarly, Raman *et al*. [34] reported a 19.39% reduction in methane production upon supplementation of buffalo sugar cane diet with SC. In this study, reductions of 11.38% and 15.85% were recorded when the animals were fed PM and CP respectively at the yeast dose level of 0.8 gSC/kg bwt.

Table 2: Effect of Saccharomyces cerevisiae on soluble residue, Neutral Detergent Fibre, Acid Detergent Fibre, Hemicellulose, Cellulose and Methane Production of WAD Sheep fed *P. maximum* and *C. pubescens*.

Parameter	Forage Diet	Treatment			
		A=0.4gSC/kgbw	B=0.6gSC/kgbw	C=0.8gSC/kgbw	D=Control
Soluble Residue (%)	PM	9.41±0.02^{a}	9.70±0.02^{b}	9.39±0.03^{a}	9.63±0.01^{c}
	CP	12.60±0.18^{a}	12.75±0.18^{a}	12.46±0.14^{ab}	11.85±0.31^{b}
NDF (%)	PM	65.91±0.47^{a}	60.72±0.37^{b}	58.51±0.13^{c}	70.69±0.44^{d}
	CP	57.38±0.14^{a}	54.27±0.15^{b}	49.63±0.31^{c}	61.63±0.61^{d}
ADF (%)	PM	32.30±0.18^{a}	29.60±0.20^{b}	27.77±0.29^{c}	35.28±0.31^{d}
	CP	27.20±0.15^{a}	25.31±0.36^{b}	24.54±0.17^{c}	29.37±0.37^{d}
Hemicellulose (%)	PM	33.62±0.55^{a}	31.12±0.31^{b}	30.73±0.21^{b}	35.41±0.47^{c}
	CP	30.18±0.21^{a}	28.95±0.22^{b}	25.09±0.39^{c}	32.26±0.54^{d}
Cellulose (%)	PM	29.93±0.22^{a}	28.02±0.25^{b}	28.12±0.30^{b}	31.61±0.23^{c}
	CP	26.84±0.14^{a}	26.17±0.21^{b}	24.60±0.18^{c}	28.45±0.22^{d}
Methane (CH_4) (mega joules/day)	PM	145.83±0.96^{a}	136.57±0.79^{b}	136.02±0.87^{b}	153.48±1.17^{c}
	CP	134.13±0.69^{a}	129.48±0.53^{b}	118.48±0.77^{c}	140.80±0.65^{d}

^{abcd}Means with different superscripts within rows are statistically different ($p < 0.05$).

The higher mitigation percentage (15.85%) reduction of methane emission with the legume (CP) treatment protocol than with the PM diet (11.38%) could be due to the higher crude protein concentration (18.95%) in CP than PM (14.53%). It is known that forages rich in protein concentrate like legumes tend to produce less methane. This assertion is in agreement with the findings of Rowlinson *et al*. [9] that diets with a high proportion of protein concentrates that promote a high propionate type of ruminal fermentation are conducive to reducing ruminal methane production although the effect on total farm green house gas emissions may be less [35]. Furthermore, the presence of plant secondary metabolite such as condensed tannins and saponins that are common in tropical legumes [36] have been shown to reduce methane production by reducing fibre digestion [37]; binding with proteins thus reducing fibre degradation of the plant protein in the rumen and through the direct inhibition of the growth of methanogens [38]. A study by Pinares-Patino *et al*. [39] reported a reduction in methane emissions by grazing sheep from 8% to 3% gross energy intake following the consumption of tannin rich forage. In a similar study [40], an extract of *Acacia mearnsii* containing 61.5% condensed tannin reduced methane emissions by 12% without decreasing fibre digestibility. Beauchemin *et al*. [41] also reported reduction in

methane emission with higher proportions of forage legumes in the diet which he attributed to lower fibre content, faster rate of passage and in some case the presence of condensed tannins.

The reduction in methane emission was accompanied by a corresponding increase in the degradability of ADF and NDF which agrees with the findings of Aka *et al*. [42] and Nasir *et al*. [43] who separately reported increased acid detergent fibre and neutral detergent fibre degradability of forages upon supplementation with SC. The higher level of methane produced in PM diet compared to CP can also be attributed to the high level of hemicelluloses and cellulose content of PM than CP. This assertion is in agreement with the report that methane production per unit of cellulose digested has been shown to be three times that of hemicellulose [28], while cellulose and hemicellulose ferment at a slower rate than non-structural carbohydrate, thus yielding more methane per unit of substrate digested [44].

In conclusion, results of this study showed that enteric methane emission from West African dwarf sheep may be mitigated by supplementation with *Saccharomyces cerevisiae* up to 0.8 gSC/kg bwt without adverse effects on the productivity of the animals fed either *Panicum maximum* or *Centrosema pubescens*.

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