Evaluation of *in vitro* gas production and nutrient digestibility of complete diets supplemented with different levels of thermotolerant yeast in Nellore rams

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Abstract

Aim: The objective of the present study was to know the effect of dietary supplementation of varied levels of thermotolerant yeast to determine best levels for sheep diets by *in vitro* gas production. An *in vivo* study on Nellore rams was used for further evaluation of diets with three best levels of yeast (obtained from *in vitro* data) to determine diet with optimum yeast level for growing lambs by assessing nutrient digestibility, plane of nutrition and nitrogen balance.

Materials and methods: A complete diet was formulated and supplemented with five levels (0 g/kg (D_1); 1 g/kg (D_2); 2 g/kg (D_3); 3 g/kg (D_4); 4 g/kg (D_5) and 5 g/kg (D_6) of thermotolerant yeast (*Saccharomyces cerevisiae,* OBV-9) @ $5x10^8$ cfu/g to determine best levels for sheep diets by IVGP technique. An *in vivo* study was conducted on Nellore rams (39.75 0.24 kg body weight, aged 3 years) in a 4 x 4 latin square design for further evaluation of diets with three best yeast levels based on *in vitro* data, to determine optimum yeast level for diets of growing lambs by assessing nutrient digestibility, plane of nutrition and nitrogen balance. The rams were housed individually in metabolic cages that allowed separation of urine and faeces to evaluate digestibility of nutrients and N balance. Animals were given 10 days adaptation period followed by 7-day collection period, feed intake and refusals were recorded. During the digestibility and N balance study, feed, refusals and faeces were analyzed for dry matter (DM), organic matter (OM) and crude protein (CP) as per AOAC, USA, while fibre fractions like neutral detergent fibre (aNDF) and acid detergent fibre (ADF) were analyzed. Data were analyzed as per the procedures suggested by Snedecor, G. W. and Cochran, W. G. (1994) and the difference between treatment means was tested for significance by Duncan's multiple-range and F Test.

Results: Higher (P<0.01) IVGP volumes, *in vitro* organic matter degradability, metabolizable energy (ME) and total degradable organic matter were recorded for rations D_2 to D_6 over control, whereas the difference among D_2 to D_4 *diets was not* significant. Significant (P<0.05) partitioning factor and efficiency of microbial biomass synthesis (EMBP) values for rations D_2 and D_3 and significant (P<0.01) microbial biomass production for D_2 to D_6 diets, recorded. Significant (P<0.01) nitrogen (N) retention was recorded in yeast supplemented rations with high EMBP than control. Dry matter intake (DMI), DMI (% b. wt.), DMI/kg $W^{0.75}$ and the digestibility of dry matter, organic matter, crude protein, crude fibre, ether extract (P<0.05), nitrogen free extract and fiber fractions were significant (P<0.01) on rations D_2 to D_4 over control, while the difference among rations D_2 to D_4 *was not* significant. All rams were in positive N balance and was higher (P<0.01) on rations D_2 to D_4 over control. The digestible crude protein (DCP) and ME were higher (P<0.01) on rations D_2 to D_4 indicating all rams were adequately met with DCP and ME requirements as suggested by ICAR, India.

Conclusions: The present study demonstrated the potential of theromotolerant *yeast* at 1 g/kg to 3g/kg level in improving digestibility of nutrients, intake of DCP and ME and N retention without affecting health of rams under study. Thus, incorporation of 1 g/kg level of thermotolerant probiotic yeast in complete diet for lambs appears to be beneficial for livestock producers.

Keywords: Nellore rams, Thermotolerant yeast, *In vitro* gas production, Digestibility, N retention.

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Introduction

Inadequate availability of feed and fodder is a major constraint to the prevalent small ruminant production system [1]. Due to population growth, grazing lands are dwindling fast because they are mostly diverted for cultivation of cereal and commercial crops to meet the urgent human needs resulting in decreased land for fodder cultivation and

Table-1. Ingredient composition of experimental complete diets

Ingredient (g/kg DM)	Complete diets with varied levels of thermotolerant yeasta					
	D1	D2	D3	D4	D5	D6
Sorghum straw	500.00	500.00	500.00	500.00	500.00	500.00
Maize	140.00	140.00	140.00	140.00	140.00	140.00
Groundnut cake	135.00	135.00	135.00	135.00	135.00	135.00
Sunflower cake	140.00	140.00	140.00	140.00	140.00	140.00
Molasses	70.00	69.00	68.00	67.00	66.00	65.00
Mineral and vitamins*	10.00	10.00	10.00	10.00	10.00	10.00
Common salt	5.00	5.00	5.00	5.00	5.00	5.00
Thermotolerant yeast	--	1.00	2.00	3.00	4.00	5.00
Feed cost/ton ($)	118.44	119.44	120.44	121.44	122.44	123.44

a. Complete diets were with (1) 0 g/kg (D_1) (2) 1 g/kg (D_2) (3) 2 g/kg (D_3) (4) 3 g/kg (D_4) (5) 4 g/kg (D_5) (6) 5 g/kg (D_6) thermotolerant yeast.
* Vitamin supplement was added @ 100 g/1000 kg complete diet.

forcing livestock to depend on alternate feed resources. Large number of feed ingredients of variable chemical composition, digestibility and nutrient quality derived from agriculture, forest, marine and industrial sources are being utilized as animal feed. Since livestock subsist mainly on poor quality roughages, several new technologies are being tried to improve their digestibility and utilization. One such effort in recent years is supplementation of yeast to livestock rations to improve the utilization of cellulosic materials, health, productivity and reproduction.

Probiotic yeast (*Saccharomyces cerevisiae*) used to enhance animal performance by improving the balance of microbial flora in the gastrointestinal tract and nutrient utilization [2]. Several studies reported changes in the stimulation of ruminal digestion in goats, cattle and sheep when fed yeast supplemented diets [3] and increased concentration of specialized bacteria associated with fiber digestion and lactic acid utilization in rumen on yeast supplementation [4,5]. But the yeast used so far, was mesophilic in nature and may not exert more beneficial action due to the harsh environmental (temperature, variation in pH and bile level) conditions under which they have to survive in the gastrointestinal (GI) tract of animal. Furthermore, it was reported that, stimulation of rumen bacteria by yeast was different with specific strains and many improvements in strain selection and stability have resulted from research in the past 15 years but strains with thermo, acid, bile and osmo tolerance need to be developed in order to explore the full potential activity of yeast culture on animal performance [6]. High temperature in the gut of animals makes the limitation of using mesophilic strain of lactobacilli as probiotic as reported [7]. Acid and bile levels in GI tract of animals vary considerably and dietary probiotic are able to survive these harsh conditions before colonizing the gut. A researcher [8] isolated thermo, acid, bile and osmo tolerant strain of yeast (S. cerevisiae, OBV-9), which grows at >42°C temperature, pH 2, 2% Ox bile and 30% sugars, that can be used as a feed additive in livestock for better productivity.

Therefore, the objective of the present study was to know the effect of dietary supplementation of varied levels of thermotolerant yeast to determine best levels for sheep diets by *in vitro* gas production. An *in vivo* study on Nellore rams was used for further evaluation of diets with three best levels of yeast (obtained from *in vitro* data) to determine diet with optimum yeast level for growing lambs by assessing nutrient digestibility, plane of nutrition and nitrogen balance.

Materials and Methods

Experiments were conducted at the Livestock Experimental Station, Livestock Research Institute, Hyderabad. The lyophilized thermotolerant yeast ($5x10^8$ cfu/g) obtained from DBT project on "Development and Application of Thermotolerant Probiotic Yeast for Enhanced Animal Productivity", College of Veterinary Science, Hyderabad was used in the present study.

Ethical approval: The study was approved by Institutional Animal Ethics Committee on 8th April, 2009.

In vitro gas production experiment: Complete diets (Table 1) supplemented with (0 g/kg (D_1); 1 g/kg (D_2); 2 g/kg (D_3); 3 g/kg (D_4); 4 g/kg (D_5) and 5 g/kg (D_6) thermotolerant yeast (*S. cerevisiae,* OBV-9) used for *in vitro* study. *In vitro* gas production (IVGP) technique [9,10] was used to describe the extent of gas

Table-2. Chemical composition and nutritive value of complete diets

Nutrient (g/kg DM)	**Complete diets with varied levels of thermotolerant yeast**					
	D1	**D2**	**D3**	**D4**	**D5**	**D6**
Dry matter	899.00	892.00	891.80	891.60	891.40	891.20
Organic matter	908.70	915.50	915.70	915.90	915.60	915.50
Crude protein	118.00	119.00	119.20	119.40	119.40	119.40
Ether extract	13.90	15.60	15.80	15.90	15.80	15.90
Crude fibre	269.80	270.90	271.20	271.40	271.20	271.20
Nitrogen free extract	507.00	510.00	509.50	509.20	509.30	509.60
Total ash	91.30	84.50	84.30	84.10	84.40	84.50
Neutral detergent fibre	547.00	565.50	565.30	565.10	565.50	565.60
Acid detergent fibre	346.30	366.30	371.20	370.00	371.20	372.40
Hemicellulose	200.70	199.20	194.10	195.10	194.30	193.20
Cellulose	278.40	281.20	282.10	285.60	285.00	285.40
Nutritive value of diets						
DCP (g/kg DM)	8.14	8.49	8.48	8.53	8.48	8.52
ME (MJ/kg DM)	8.78	9.57	9.53	9.58	9.53	9.54

production from treatment diets. Rumen liquor was obtained with the help of a stomach tube fitted with vacuum pump from Nellore rams that were fed complete diets before offering the morning feed. Approximately 350 ml of rumen liquor was siphoned from different depths and directions of reticulo rumen and transferred into pre heated thermos flask, strained through a fourfold muslin cloth and flushed with CO_2. Rumen fluid–medium mixture (inoculum) is prepared under continuous flushing with Co_2.

Three replications of 200 mg dry weight of feed samples were weighed into 100 ml calibrated syringes and incubated with 30 ml of mixed rumen inoculum at 39°C with parallel incubation of blanks [11]. Undigested residues were recovered and freeze dried for 6 h and oven dried at 60°C for 24 h and weighed to determine apparent undigested residue, which was extracted in 100 ml of NDS by boiling for one hour, followed by filtration on preweighed gooch crucibles, and washing in hot distilled water and acetone to recover true undigested residue [12]. Crucibles with undigested residue were dried at 100°C overnight and weighed to determine true undigested residue. Residue was ashed at 500°C for 3 h to determine true undigested OM, which was corrected for the appropriate blanks. The TDOM was calculated as the difference between OM incubated and the undigested OM recovered in the residue of ND extraction.

Determination of the truly undigested substrate reveals the amount of substrate that was totally available to fermentation and the gas volume indicates the proportion of this substrate used for the SCFA. A variation in the relationship reflects the variation in microbial yield per unit SCFA produced [13]. This variation can be expressed as Partitioning factor (PF), which is calculated as the ratio of substrate truly degraded to gas volume produced [14]. The Efficiency of microbial biomass production (EMBP) of experimental rations was determined by measuring the ratio of TDOM and gas production as described [15].

In vivo experiment

Experimental design, animals and feeding: A "4 x 4 Latin Square Designed digestion trial" was conducted on four Nellore rams (39.75 0.24 kg body weight, aged 3 years) chosen randomly from the Sri Venkateswara Veterinary University farm. Test diets with three best performing levels based on *in vitro* data (D_2, D_3 and D_4) were further evaluated by *in vivo* study. These diets were formulated to have 12% CP (DM basis) to meet requirements of animals according to [16]. Diets were mixed biweekly during the study and were sampled upon mixing to ensure consistency in their chemical composition (Table 2). Animals were kept in hygienic, well ventilated individual metabolic cages with feeding and watering arrangement during the trial. Rams were injected with ivermectin (Ivectin 1%) for treatment and control of GI and ecto-parasites. Animals were shifted to metabolic cages that allowed separate collection of urine and faeces to evaluate digestibility of nutrients and N balance, 10 days prior to collection period to acclimatize the cage environment. Animals were offered weighed quantities of respective diets and clean drinking water *ad libitum* for the duration of the study. A 10 d preliminary period and a 7 d collection period was followed during the digestion trial. During the collection period, feed intake and refusals were recorded, feed samples and refusals were sampled for further analysis. Daily faecal output was collected, Table-3. Least square means for *in vitro* gas production (IVGP) profiles of test diets

IVGP profiles	Complete diets with varied levels of thermotolerant yeast						SEM
	D1	D2	D3	D4	D5	D6	
IVGP (ml/200 mg DM)	45.50[a]	49.33[b]	50.00[bc]	50.50[bc]	51.00[c]	51.50[c]	0.51
IVOMD (mg)	100.10[a]	108.53[b]	110.00[bc]	111.10[bc]	112.20[c]	113.30[c]	1.11
ME (MJ/kg DM)	8.39[a]	8.91[b]	9.00[bc]	9.07[bc]	9.14[c]	9.20[c]	0.07
TDOM (mg)	142.80[a]	161.22[b]	162.83[bc]	163.38[c]	162.64[bc]	163.04[bc]	1.81
PF (mg/ml)*	3.14[a]	3.27[b]	3.26[b]	3.24a[b]	3.19[ab]	3.17[ab]	0.02
MBP (mg)	42.70[a]	52.68[b]	52.83[b]	52.28[b]	50.44[b]	49.74[b]	0.96
EMBP (g/kg)*	299.00[a]	326.79[b]	324.42[b]	319.97[ab]	310.13[ab]	304.94[ab]	3.57

a, b, c : means with different superscripts row wise differ significantly (P<0.01); *(P<0.05)

weighed and recorded, and then 10% was kept for subsequent analyses. Similarly using glass bottles, urine was collected, weighed and recorded, and then 5% was aliquoted for N estimation. Each bottle had 50 ml of 6N HCl to prevent nitrogen losses. All samples were dried at 55 °C in a forced-air oven to reach a constant weight, air equilibrated, and then ground to pass 1 mm screen and stored in air tight bottles for further analysis.

The feed and faecal samples were analysed for proximate constituents [17] and fibre fractions [12]. *In vitro* gas production (ml/200 mg DM) was measured as per the formula specified [10,18]. Metabolizable energy (ME, MJ/kg DM) value was calculated from total digestible nutrients (TDN) using the factors suggested [19]. Data on *in vivo* and *in vitro* studies were analysed according to the procedures suggested [20] and the difference between treatment means was tested for significance by Duncan's multiple-range and F Test [21].

Results and Discussion

In vitro gas production (IVGP) profiles: There was a significant (P<0.01) difference in gas production for control and test diets (D_2 to D_6), and the difference was not significant among diets with yeast level up to 3 g/kg, whereas the difference was significant (P<0.01) with diets D_5 and D_6 (Table 3), showing the use of dietary yeast in sheep rations for desired effects. These results were in agreement with [22], who reported, total gas was increased with the yeast supplementation that might have resulted from the increased production of propionate, because carbon dioxide is produced when propionate is made by some ruminal bacteria via the succinate to propionate pathway. A researcher [23] also reported methane production decreased linearly, whereas hydrogen accumulation decreased quadratically after 24 h with increasing yeast concentration. The difference in gas production for test diets in current study might be due to the suppressing effect of high cell wall and lignin present in feeds resulting in decreased attachment of ruminal microbes to feed particles [24]. Gas production is basically the result of fermentation of carbohydrates to acetate, propionate and butyrate and gas from protein is relatively small, compared to carbohydrate fermentation [11]. Higher gas values obtained for the diets D_2 to D_6, indicating a better nutrient availability for rumen microorganisms [25]. These results were also in agreement with the reports of [5]. Higher (P<0.01) IVOMD, ME and TDOM were recorded for yeast supplemented diets compared to D_1, and there was no difference among diets with yeast level up to 3 g/kg, whereas the difference was significant (P<0.01) with D_5 and D_6 diets except for TDOM. Higher (P<0.01) IVOMD, ME and TDOM values recorded for yeast supplemented diets, indicating addition of yeast stimulated microbial metabolism and content of fermentable carbohydrates and available nitrogen, i.e. a better nutrient availability for rumen micro-organisms [24-26]. The findings of current study were in agreement with the results of [27,28], who reported higher IVOMD values on yeast based rations. IVOMD values recorded were higher than the in vivo OMD values of the present study, possibly due to differences in rumen fluid content in which the test feeds were incubated.

Similarly, higher (P<0.05) PF and EMBP for diets D_2 and D_3, and higher (P<0.01) MBP for yeast supplemented diets were recorded in the current study. There was no difference for PF and EMBP among diets that contain yeast level up to 3 g/kg, whereas the difference was lower (P<0.05) for D_5 and D_6 diets, which were also comparable with control, while there was no difference observed for MBP among yeast supplemented diets. Higher PF, EMBP (P<0.05) and MBP (P<0.01) values recorded for yeast supple-

Table-4. Least square means for DMI, nutrient digestibility and plane of nutrition in rams fed test diets

Parameter	Complete diets with varied levels of thermotolerant yeast				
	D1	D2	D3	D4	SEM
DM intake					
Body weight (kg)	39.50	39.50	40.50	39.50	0.24
DM intake (g/d)	1033.67[a]	1148.09[b]	1161.19[c]	1159.08[bc]	13.85
DM intake (% b. wt.)	2.62[a]	2.91[b]	2.87[b]	2.93[b]	0.04
DM intake/kgW0.75 (g)	65.61[a]	72.90[b]	72.36[b]	73.57[b]	0.86
Nutrient digestibility (%)					
Dry matter	52.60[a]	57.36[b]	58.12[b]	60.28[b]	0.90
Organic matter	58.30[a]	62.32[b]	62.73[b]	64.40[b]	0.77
Crude protein	68.97[a]	71.35[b]	71.12[b]	71.41[b]	0.35
Ether extract*	71.24[a]	75.89[b]	75.11[b]	75.77[b]	0.67
Crude fiber	58.99[a]	62.94[b]	62.89[b]	62.93[b]	0.52
Nitrogen free extract	58.01[a]	61.92[b]	61.53[b]	61.95[b]	0.56
Neutral detergent fiber	56.09[a]	62.55[b]	63.96[b]	65.23[b]	1.07
Acid detergent fiber	49.37[a]	58.51[b]	59.03[b]	62.05[b]	1.43
Hemicellulose	65.24[a]	70.20[b]	73.05[b]	71.82[b]	1.00
Cellulose	51.36[a]	59.73[b]	59.64[b]	60.42[b]	1.10
Plane of nutrition					
DCP intake (g/d)	84.13[a]	97.47[b]	98.44[b]	98.83[b]	1.63
DCP intake/kgW0.75 (g)	5.34[a]	6.19[b]	6.11[b]	6.27[b]	0.10
ME intake (MJ/d)	9.07[a]	10.98[b]	11.07[b]	11.11[b]	0.23
ME intake/kgW0.75 (MJ)	0.58[a]	0.70[b]	0.69[b]	0.71[b]	0.01

1. Complete diets were with (1) 0 g/kg (D_1) (2) 1 g/kg (D_2) (3) 2 g/kg (D_3) (4) 3 g/kg (D_4) thermotolerant yeast.
a, b, c : means with different superscripts row wise differ significantly (P<0.01); *(P<0.05).

mented diets compared to D_1, indicating higher feed intake. Similarly, higher (P<0.01) *in vivo* DMI recorded for the diets having higher PF in the present study, is consistent with the reports of [29,30], who defined PF as 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.* In the current study, diets having higher IVGP volumes were having a low MBP value, showing an inverse relationship between IVGP and MBP. The results obtained were also in agreement with the findings of [13]. The intake of aNDF and ADF recorded in Nellore rams in digestibility study was lowest for the rations having lowest PF values, and these results were also consistent with findings of [30]. The average efficiency of microbial biomass production (EMBP, g/kg) from present study indicated, different contents of fermentable carbohydrates and available nitrogen to the rumen microbes. The *in vivo* digestibility study on rams has also shown higher (P<0.01) nitrogen retention for the diets with yeast over control with higher EMBP.

In addition to chemical analysis, the IVGP technique applied in the present study proved to be simple to determine the difference in IVGP, OMD and ME of diets with yeast, which can be more rapidly applied in developing countries because of its convenience, cost saving and practical applicability [31]. Further, diets producing higher ME and OMD were having potential to be used as protein and energy supplements for ruminants as reported by [32], though, the predictive ME values (8.78 to 9.58) were found within the range of reported values for a large number of feedstuffs [33]. It is opined that, addition of yeast up to 3 g/kg level in complete diet is appropriate for adult sheep, as the difference between 1 g/kg to 5 g/kg level of yeast was meager.

Nutrient digestibility and plane of nutrition: Least square means of DM intake, digestibility of nutrients and plane of nutrition of rams fed complete diets with 1 g/kg to 3 g/kg level of thermotolerant yeast are presented in (Table-4). Higher (P<0.01) DMI, DMI (% b. wt.) and DMI/kg$W^{0.75}$ were recorded in rams fed yeast supplemented diets, might be due to more intake of diets and might also due to yeast. Although differences existed in the acceptability of

Table-5. Least square means for N retention in rams fed test diets

N retention	Complete diets with varied levels of thermotolerant yeast				
	D1	D2	D3	D4	SEM
N intake (g/d)	20.12[a]	22.22[b]	22.02[b]	22.22[b]	0.25
N balance (g/d)	7.32[a]	9.82[b]	9.16[b]	9.34[b]	0.28
N balance (% intake)	36.37[a]	44.21[b]	41.55[b]	42.02[b]	0.91
N balance (% absorbed)	52.74[a]	61.96[b]	58.41[b]	58.80[ab]	1.07

a, b : means with different superscripts row wise differ significantly (P<0.01).

experiential diets, all the animals met with DMI requirements as suggested by [16] indicating, these test diets were adequately palatable (due to blending of roughages and concentrates in correct proportions) and inclusion of yeast might have influenced the DMI, on par with [34] recommendations for adult sheep. These results were in accordance with the findings of [35-39] in rams and [3] in goats on yeast based complete diets.

Digestibility of DM, OM, CP, EE (P<0.05), CF, NFE, NDF, ADF, Hemicellulose and Cellulose was higher (P<0.01) on yeast supplemented diets, with no difference among them. Higher DM digestibility was also reported by [35,40,41,42] with dietary addition of yeast in *sheep. H*igher digestibility of OM and CP by feeding yeast supplemented diets was reported by [40-44] *in sheep* and [3] in goats. The results indicated that, yeast supplementation might have exerted selective stimulatory effect on specific rumen bacteria responsible for fibre degradation and microbial protein synthesis in rams [45,46]. The EE digestibility was increased by around 3.5-5.0 % in yeast supplemented diets, than control. Higher EE digestibility on complete diets in Barbari goats was also reported [47]. Significantly higher CF digestibility on yeast supplemented diets might be due to complementary effect of yeast by providing favourable rumen environment for stimulating cellulolytic and hemicellulolytic bacteria along with concentrates. The results were consistent with findings of [35,41,42,48], who reported significant (P<0.05) improvement in CF digestibility in sheep and [3] in goats fed feeds with yeast. The results were also in accordance with [9], who reported higher CF digestibility with dietary yeast supplementation *in calves.* NFE digestibility was increased by 3.85 - 5.01 percentage units on yeast supplemented diets than control. Improved efficiency in feed utilization, better CF digestion and higher DM digestibility might have resulted in significantly higher NFE digestibility. The results were in agreement with the findings of [41,42] on yeast supplemented diets in sheep.

The increased aNDF digestibility recorded in the present study corroborate with the findings of [40], *who* reported similar results on dietary incorporation of yeast *in sheep*. increased aNDF degradability due to addition of yeast in rations under incubation trials was reported [49,50]. On contrary, [51,52] reported no difference in digestibility of aNDF, when sheep fed rations with or without yeast. Higher (P<0.01) ADF digestibility for yeast supplemented diets, recorded in the present study was in agreement with findings of [39] in cows and [53] in buffalo calves, who reported increased ADF digestibility due to dietary supplementation of yeast, whereas [54] recorded no impact of yeast addition in diets on ADF digestibility in sheep. Higher hemicellulose and cellulose digestibility recorded in the present study was consistent with findings of [53], who observed higher (P<0.05) hemicellulose digestibility due to dietary supplementation of yeast in buffalo calves.

Similarly, higher (P<0.01) intake and intake per unit/kg$W^{0.75}$ of DCP and ME was also recorded in the current study on three test diets over control, might be due to higher energy density as well as higher digestibility of CP and other nutrients. Further, all the rams were adequately met with the DCP and ME requirements as suggested by [16]. The results of the present study were in agreement with findings of [41], who reported higher (P<0.05) DCP intake in sheep fed yeast based complete diets. Higher (P<0.05) ME values in calves fed diets with different levels of thermotolerant yeast was also reported by [53].

Nitrogen balance: The N intake, N balance (% intake) and N balance (% absorbed) of yeast supplemented diets were significantly (P<0.01) higher than control, while there was no difference recorded among these diets (Table 5). Higher daily average N intake and balance on yeast supplemented diets, reflecting the daily average intake and digestibility pattern of CP among the test diets. Higher N balance on these rations compared to control might be due to addition of thermotolerant yeast. Positive N balance was recorded in all test diets indicating, all the rations met with the N requirements of sheep, might be because of optimum utilization of dietary N by microbes due to matching supply of energy [55]. Positive nitrogen balance in sheep fed complete diets was reported by [56]. The results of the present study were consistent with the findings of [57], who reported N degradability and N balances were increased by the addition of yeast in sheep. Higher (P<0.05) N retention in sheep fed yeast based complete diets was also reported by [41].

Conclusion

The results of the *in vitro* gas production study indicated that the theromotolerant yeast level up to 3 g/kg can be incorporated in adult sheep rations and in vivo study on Nellore rams demonstrated the potential for supplementing yeast at 1 g/kg level without much economic impact for improvement in digestibility of nutrients, intake of DCP and ME and positive N balance without any effect on the health of sheep. Thus, incorporation of 1 g/kg level of thermotolerant probiotic yeast in the straw based complete diet shall be recommended for economical rearing of ram lambs with fattening diets that may be beneficial for livestock producers.

Author's Contribution

Implementation of study design**:** Ch. Harikrishna, M. Mahender, Y. Ramana Reddy and M. Gnana Prakash, Data recording and analysis: Ch. Harikrishna and M. Pavani, Drafting of manuscript: Ch. Harikrishna, Revision of manuscript: Ch. Harikrishna, M. Mahender, Y. Ramana Reddy, M. Gnana Prakash and K. Sudhakar. All author read and approved the final manuscript.

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Competing interest

Authors declare that they have no conflict of interest.

References

1. Anbarasu, C., Dutta, N., Sharma, K. and Rawat, M. (2004). Response of goats to partial replacement of dietary protein by a leaf meal mixture containing *Leucaena leucocephala, Morus alba* and *Tectona grandis*. *Small Rumin. Res.* 51: 47-56.
2. Moallem, U., Lehrer, H., Livshitz, M., Zachut, M. and Yakoby, S. (2009). The effects of live yeast supplementation to dairy cows during the hot season on production, feed efficiency and digestibility. *J. Dairy Sci.* 92: 343-351.
3. Rohilla, P. P., Patil, N. V. and Bohra, H. C. (2009). Effects of probiotics and nutri mix on kids and goats. *Indian Vet. J.* 86: 640-641.
4. Khadem, A. A., Pahlavan, M., Afzalzadeh, A. and Rezaeian, M. (2007). Effects of live yeast *S. cerevisiae* on fermentation parameters and microbial populations of rumen, total tract digestibility of diet nutrients and on the *in situ* degradability of alfalfa hay in Iranian Chall sheep. *Pakistan J. Biol. Sci.* 10(4): 590-597.
5. Malik, R. and Singh, R. (2009). Effect of yeast and fungi culture on in vitro ruminal fermentation. Indian *J. Anim. Nutri*. 26(1): 40-45.
6. New Bold, C. J., Wallance, R. J. and McIntosh, F. M. (1995). Different strains of *Saccharomyces cerevisiae* differ in their effects on ruminal bacterial numbers *in vitro* and in sheep. *J. Anim. Sci*. 73: 1811-1818.
7. Lankaputra, W. E. V. and Shah, N. P. (1995). Cultured dairy products. *Tamil Nadu J. Anim. Vet. Sci.* 30: 2-7.
8. Bhima, B., Reddy, M. S., Pavana Jyothi, Ch., Reddy, Y. R. and Rao, L. V.(2008a). Optimization of conditions for large scale production of thermotolerant probiotic yeast (*S. cerevisiae*) by Taguchi methodology. Proceedings of the 3rd International Congress on Bioprocesses in Food Industries: Abstr. IL 12.
9. Menke, K. H., Raab, L., Salewski, A., Steingass, H., Fritz, D. and Schneider, W. (1979). Estimation of the digestibility and metabolizable energy content of ruminant feeding stuffs from the gas production when they are incubated with rumen liquor. *J. Agri. Sci*. 93: 217-222.
10. Menke, K. H. and Steingass, H. (1988). Estimation of energetic feed value obtained from chemical analysis and *in vitro* gas production using rumen fluid. *Anim. Res. Dev*. 28: 7-55.
11. Makkar, H. P. S., Blummel, M. and Becker, K. (1995). Formation of complexes between polyvinyl pyrrolidones or polyethylene glycols and tannins and their implications in gas production and true digestibility in *in vitro* techniques. *Br. J. Nutri.* 73: 897-933.
12. Van Soest, P. J., Robertson, J. B. and Lewis, B. A. (1991). Methods for dietary fiber, neutral detergent fiber and non starch polysaccharides in relation to animal nutrition. *J. Dairy Sci*. 74: 3583-3597.
13. Blummel, M., Steingas, H. and Becker, K. (1997a). 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. Nutri. 77:* 911-921.

14. Blummel, M., Steingas, H. and Becker, K. (1994). The partitioning of *in vitro* fermentation products and its bearing for voluntary feed intake. *Proceedings of the Society of Nutrition Physiology 3:* Abstr. 123.
15. Blummel, M., Makkar, H. P. S. and Becker, K. (1997b). *In vitro* gas production: a technique revisited. *J. Anim. Physiol. Anim. Nutri*. 77: 24-34.
16. ICAR. (1998). *Nutrient requirements of livestock and Poultry*, ICAR, Pusa, New Deli.
17. AOAC (1997). Association of Official Analytical Chemists, *Official Methods of Analysis*. 16th edition, Maryland, USA.
18. Krishnamoorthy, U., Soller, H., Steingass, H. and Menke, K. H. (1991). A comparative study on rumen fermentation of energy supplement *in vitro*. *J. Anim. Physiol. Anim. Nutri*. 65: 28-35.
19. NRC. (1985). *Nutrient requirements of sheep* (6th edition). National Academy of Science, National Research Council, Washington DC, USA.
20. Snedecor, G. W. and Cochran, W. G. (1994). *Statistical methods.* 8th edn, Iowa State University Press, Ames, Iowa, USA-50010.
21. Duncan, D. B. (1955). Multiple range and multiple F tests. *Biometrics* 11: 1-42.
22. Wolin, M. J. and Miller, T. L. (1988). Microbe-microbe interactions. In: *Rumen Microbial Ecosystem*. P.N. Hobson, ed. Elsevier Science, Essex, U.K. Page: 343.
23. Lila, Z. A., Mohammed, N., Yasui, T., Kurokawa, Y., Kanda, S. and Itabashi, H. (2004). Effects of a twin strain of *S. cerevisiae* live cells on mixed ruminal microorganism fermentation *in vitro*. *J. Anim. Sci*. 82: 1847-1854.
24. Paya, H., Taghizadeh, A., Janmohammadi, H. and Moghadam, G. A. (2007). Nutrient digestibility and gas production of some tropical feeds used in ruminant diets estimated by the *in vivo* and *in vitro* gas production techniques. *Amer. J. Anim. Vet. Sci.* 2(4): 108-113.
25. Ahmed, G. M. and Abdel, N. M. A. F. E. (2007). Chemical composition and *in vitro* gas production characteristics of six fodder trees, leaves and seeds. *Res. J. Agri. Biol. Sci.* 3(6): 983-986.
26. Khanum, S. A., Yaqoob, T., Sadaf, S., Hussain, M. and Jabbar, M. A. (2007). Nutritional evaluation of various feedstuffs for livestock production using *in vitro* gas method. *Pakistan Vet. J.* 27(3): 129-133.
27. Oeztuerk, H., Schroeder, B., Beyerbach, M. and Breves, G. (2005). Influence of living and autoclaved yeasts of *Saccharamyces boulardii* on *in vitro* ruminal microbial metabolism. *J. Dairy Sci*. 88(7): 2594-2600.
28. Olson, K. C., Caton, J. S., Kirby, D. R. and Norton, P. L. (1994). Influence of yeast culture supplementation on steers grazing mixed grass prairie in northern great plain. I. Dietary composition intake and *in situ* nutrient disappearance. *J. Anim. Sci*. 72: 2149-2157.
29. Blummel, M., Karsli, A. and Russel, J. R. (2003). 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.* 90: 1-11.
30. Thirumalesh, T. and Krishnamoorthy, U. (2009). Effect of feeding diets differing in Partitioning factor (PF) on intake, digestibility and nitrogen metabolism in ram lambs. *Anim. Nutr. Feed Technol*. 9: 11-20.
31. Kamalak, A., Canbolat, O., Kizilsimsek, M., Ozkan, C. O. and Ozkose, E. (2005). Effect of variety on chemical composition, *in vitro* gas production, metabolizable energy and organic matter digestibility of alfalfa hays. *Live. Res. Rural Dev.* 17(7) http://www.cipav.org.co/lrrd/lrrd17/7/kama16044.htm.
32. Babeyami, O. J., Hamzat, R. A., Bamikole, M. A., Anurudu, N. F. and Olomola, O. O. (2006). Preliminary studies on spent tea leaf: in vitro gas production as affected by chemical composition and secondary metabolites. *Pak. J. Nutr*. 5(5): 497-500.
33. Krishnamoorthy, U., Soller, H., Steingass, H. and Menke, K. H. (1995). Energy and protein evaluation of tropical feed stuffs for whole tract and ruminal digestion by chemical analysis and rumen inoculum studies *in vitro*. *Anim. Feed Sci. Technol*. 52: 177-188.
34. Kearl, L. C. (1982). *Nutrient requirements of ruminants in developing countries.* International Feedstuffs Institute, Utah State University, Logan Utah, U.S.A.
35. Abd El Hafez, G. A., Daghash, H. A. and Kobeisy, M. A. (1997). Growth performance and some blood constituents in sheep fed sugarcane tops or bagasse treated with urea. *Egyptian J. Nutr. Feeds* (1997 Nov. Special): 161-171.
36. Gonzalez, S. S. (1993). Improving utilization of poor quality forages with yeast culture. *Biotechnology in Feed Industry* pp: 255-267.
37. Plata, P. F. X., Ricalde, V. R., Melgoza, C. L. M., Lara, B. A., Aranda, I. E. and Mendoza, M. G. D. (2004). Effects of monensin and yeast (*S. cerevisiae*) treatment on sheep performance. *Revista Cientifica, Facultad de Ciencias Veterinarias, Universidad del Zulia* 14: 522-525.
38. Reddy, N. M., Reddy, G. V. N. and Reddy, M. R. (1994). Utilization of fodder based complete diets in crossbred bulls. *Indian J. Anim. Sci*. 64: 631–635.
39. Wohlt, J. E., Corcine, T. J. and Zajac, P. K. (1998). Effect of yeast on feed intake and performance in cows fed diets based on corn silage during early lactation. *J. Dairy Sci.* 81: 1345-1352.
40. Haddad, S. G. and Goussous, S. N. (2005). Effect of yeast addition on nutrient intake, digestibility and growth performance of Awassi lambs. *Anim. Feed Sci. Technol*. 118: 343-348.
41. Mahender, M., Prasad, V. L. K. and Reddy, G. V. N. (2006). Effect of yeast culture on growth and nutrient utilization in Nellore lambs. *Indian J. Anim. Nutr*. 23(1): 10-13.

42. Salem, F. A., Soliman, A. S., Abdelmawla, S. M. and El Mahdy, M. R. M. (2000). Effect of some feed additives to rations of growing sheep on growing performance, rumen fermentation, blood constituents and carcass characteristics. *Annals Agri. Sci. Moshtohor* 38: 1885-1904.
43. Reddy, M. S., Reddy, M. R. and Reddy, G. V. N. (1990). Effect of particle size on physical characteristics nutrient utilization of groundnut hulls on complete diet of sheep. *Indian J. Anim. Sci*. 60: 455-459.
44. Sohn and Song (1996). Effect of feeding yeast diets on the ruminal fermentation characteristics and whole tract digestibility by sheep. *Korean J. Anim. Sci*. 38: 578–588.
45. Erasmus, L. J. (1991). The importance of the duodenal amino acid profile for dairy cows and the impact of changes in profiles following use of yea sacc[1026]. *Nutr. Abstr. Reviews* 61: 5134.
46. Rossi, F. P. S., Cocconcelli and Masoero, F. (1995). Effect of a *S. cerevisiae* culture on growth and lactate utilization by ruminal bacterium *Megashaera elsdenii*. *Annual Zootechnia* 44: 403.
47. Samantha, A. K., Singh, K. K., Das, M. M., Maity, B. B. and Kundu, S. S. (2003). Effect of complete feed block on nutrient utilization and rumen fermentation in Barbari goats. *Small Rumin. Res*. 48: 95–102.
48. Salem, F. A., El Mahdy, M. R., Zaza, E. H. and Abo Donia, F. M. A. (2002). Effect of non-hormonal growth promoters on digestibility, rumen fermentation, some blood constituents and growth in sheep. *Annals Agri. Sci. Moshtohor* 40: 1449-1460.
49. Carro, M. D., Lebzien, P. and Rohr, K. (1992). Influence of yeast culture on the *in vitro* fermentation (Rusitec) of diets containing variable portions of concentrates. *Anim. Feed Sci. Technol*. 37: 209-220.
50. Mpofu, I. D. T. and Ndlovu, L. R. (1994). The potential of yeast and natural fungi for enhancing fibre digestibility of forages and roughages. *Anim. Feed Sci. Technol*. 48: 39-47.
51. Arcos Garcia, J. L., Castrejon, F. A., Mendoza, G. D. and Perez, G. E. P. (2000). Effect of two commercial yeast cultures (*S. cerevisiae)* on ruminal fermentation and digestion in sheep fed sugar cane tops. *Livest. Prod. Sci.* 63: 153–157.
52. Kawas, J. R., Garcia, C. R., Fimbres, D. H., Garza, C. F., Hernandez, V. J. F. G., Olivares, S. E. and Lu, C. D. (2007). Effects of sodium bicarbonate and yeast on nutrient intake, digestibility and ruminal fermentation of lambs fed finishing diets. *Small Rumin. Res.* 67: 149-156.
53. Bhima, B., Reddy, Y. R., Pavani, M., Reddy, M. S. and Rao, L. V. (2008b). Effect of different levels of thermotolerant probiotic yeast on intake and nutrient utilization in Murrah buffalo steers fed straw based complete diets. *Proceedings of the 3rd International Congress on Bioprocesses in Food Industries*: Abstr. IL 27.
54. Andrighetto, I., Bailoni, L., Cozzi, G. and Berzaghi, P. (1993). Effects of yeast culture addition on digestion in sheep fed a high concentrate diet. *Small Rumin. Res.* 12(1): 27-34.
55. Reddy, G. V. N. and Reddy, M. R. (1991). Effect of processing cotton seed hulls on nutrient utilization in sheep and goats. *Indian J. Anim. Nutri*. 8: 123-126.
56. Tagel Din, A. E., Nour, A. M., Abou Akkada, A. R. and Zahran, S. M. (1990). Effect of source of roughage on nutrients value of complete diets. *Indian J. Anim. Sci.* 60: 233 – 235.
57. El Waziry, A. M., Kamel, H. E. M. and Yacout, M. H. M. (2000). Effect of baker's yeast (*S. cerevisiae*) supplementation to Berseem hay diet on protein digestion and rumen fermentation of sheep. *Egyptian J. Nutri. Feeds* 3: 71-82.

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