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Influence of Stress-Resistant Yeast Culture (OBV9) Supplementation on the Productive Performance of Water Buffalo

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ABSTRACT

The inclusion of yeast (*Saccharomyces cerevisiae*) in animal feeds as a probiotic is well recognized in the livestock industry, however, the effects on animal performance is inconsistent and depends on the type of strain used and the ability to be viable in the gut. Recently, we have isolated a stress-resistant yeast culture strain (OBV9) to tolerate and be effective in the rumen environment. The objectives of this study were (1) To determine the concentration of the optimum level of yeast to be included in the animal feed and (2) To evaluate the effect of total mixed rations with and without the supplementation of two different (mesophilic and stress-resistant) strains of yeast (*Saccharomyces cerevisiae*) on the productive performance of lactating murrah buffaloes. An *in vitro* anaerobic fermentation study was carried out with 0.0, 1.0, 2.0, 3.0, 4.0 and 5.0 g of the yeast culture strain OBV9 using both Tilley and Terry method and also Menke's *in vitro* gas production method. Both methods indicated that 1.0 g kg^{-1} concentration of yeast culture favored microbial biomass production, DM digestibility and also *in vitro* gas production. A subsequent *in vivo* experiment was conducted for 180 day with 18 lactating buffaloes (Murrah breed) which were randomly assigned to three treatment groups based on yeast culture supplementation (diet 1-control, diet 2-mesophilic yeast culture and diet 3-stress-resistant yeast culture). Measurements on voluntary feed intake and milk composition were recorded daily. Samples for metabolic measurements were taken over a fifteen day period towards the end of the experiment. Results indicated that stress-resistant yeast culture supplementation increased the digestibilities of dry matter, hemicellulose and cellulose ($p<0.05$). Although, the total milk production remained similar between the diets, supplementation of yeast culture increased both the milk protein and somatic cell counts ($p<0.05$), while stress-resistant yeast culture also increased fat content in the milk ($p<0.06$). It is concluded that stress-resistant yeast supplementation at the rate of 1 g kg^{-1} of DM to lactating buffaloes had a positive impact on milk production.

Key words: Stress-resistant yeast, total mixed ration, lactating buffaloes, nutrient digestibility, milk production

INTRODUCTION

Supplementation of *Saccharomyces cerevisiae* to cattle had a positive influence on productive performance of dairy cattle (Newbold *et al*., 1996). It was reported that the supplementation of yeast culture to animals directly influences the rumen microbial populations and contribute to an increase in fiber digestibility (Kamra *et al*., 2002). The yeast culture is a rich source of growth factors, promoters and vitamins which are required for the growth and multiplication of microbes in the rumen and by doing so bring about a balance in the ruminal pH (Williams *et al*., 1991). Further, the yeast culture also has the ability to remove oxygen and maintain homeostasis in the rumen by promoting the growth of lactate utilizers (El-Waziry and Ibrahim, 2007).

The supplementation of yeast culture *Saccharomyces cerevisiae*, strain SC-47 to lactating Holstein dairy cows resulted in an increased milk yield (Nikkhah *et al*., 2004) and when included at 10 g ha day^{-1} increased both milk yield and butter fat (Hugues, 1987). Yeast culture supplementation also increased C_{14} and C_{16} fatty acids of milk fat (Kholif *et al*., 2000), in lactating buffaloes. However, the positive influence of yeast supplementation on animal production was not consistent across several reports (Kumar *et al*., 1992; Kamalamma *et al*., 1996; Salama *et al*., 2002). Ideally, pH around 4.5-5.5 and temperature at 30°C will favor the growth of active *Saccharomyces cerevisiae* yeast culture (Lopez, 2000). Inconsistencies between reports on the effect of yeast supplementation on animal performance is possibly due to the difference in the type of yeast culture strain used and its concentration as probiotic in animal feeds. Also, the efficacy of yeast supplementation was dependent on the strain's ability to tolerate diverse conditions such as high temperature (Kamra, 2005) and the high concentrations of pressure and volatile fatty acids in the rumen (Lankaputhra and Shah, 1995; Jin *et al*., 1998).

Considering these inconsistencies on the effect of yeast supplementation on animal production, we have isolated a yeast culture strain that is stress-resistant (strain OBV9) to tolerate and be effective in the rumen environment. Complete characterization of the strain including sequencing of 5.8S rRNA and Internal Transcribed Spacer (ITS 1 and 2), revealed that this strain is 99% similar to *Saccharomyces cerevisiae* (Bhima *et al*., 2010). The purpose of this study is to evaluate the effectiveness of this yeast culture strain OBV9 as a probiotic on the productive performance of lactating water buffaloes. The objectives were (1) To determine the concentration of the optimum concentration of yeast culture supplement to be included in the animal feed using *in vitro* experiments and (2) To evaluate the effect of total mixed rations with and without the supplementation of both stress-sensitive and stress-resistant strains of yeast (*Saccharomyces cerevisiae*) on the productive performance of lactating murrah buffaloes.

MATERIALS AND METHODS

Selection of yeast culture (*Saccharomyces cerevisiae*) strains

Mesophilic strain: Yeast *Saccharomyces cerevisiae* NCIM 3190 which was used as probiotic for animal feed supplementation by Mahender *et al*. (2005) and Reddy and Bhima (2003) was obtained from microbial culture collection centre, National Chemical Laboratory, Pune, India.

Stress-resistant strain: This strain was isolated from brewery effluents, screened for its growth under different stress conditions such as temperature and pH present in the rumen. It was characterized as OBV9 strain of *Saccharomyces cerevisiae* with 99% similarity (NCBI-GENEBANK accession number: GU229793) by sequencing its 5.8S rRNA and internal transcribed spacer 1 and 2 (Bhima *et al*., 2010). The difference in growth conditions of both strains of *Saccharomyces cerevisiae* OBV9 and NCIM3190 were furnished in Table 1.

Table 1: Growth conditions of stress-resistant yeast *Saccharomyces cerevisiae* OBV9 and mesophilic yeast *Saccharomyces cerevisiae* NCIM3190

Growth parameters	OBV9	NCIM3190
Temperature (°C)	39.0±2	30.0±2
pH	2.0	5.5
Organic acid mixture (%) (Acetic: Butyric: Propionic 70:20:10)	1.5	0.25
Sugar (%) (osmotic pressure)	30.0	5.0
Ox bile (%)	1.0	0.25

***In vitro* study:** Three rumen-fistulated steers fed with sorghum straw based total mixed ration were selected as the donor animals for ruminal fluid. The steers were cared for in accordance with the Guide for the Care and Use of Laboratory Animals prepared by Tokyo University of Agriculture and Technology and maintained (Animal experimental station, Department of Animal Nutrition, Rajendra Nagar, Hyderabad). Before the morning feeding, approximately 500 mL of ruminal fluid was drawn from each of the buffalo and deposited into a vacuum flask that had been previously flushed with O_2-free CO_2, mixed and immediately transported to the laboratory. The mixed rumen sample was strained through four layers of surgical gauze into an Erlenmeyer flask under continuous flushing with CO_2 and efforts were made to maintain the temperature at 38°C. This rumen fluid was used as the inoculum for *in vitro* evaluation.

The study involved sorghum straw based total mixed ration as the substrate which was supplemented with 0.0, 1.0, 2.0, 3.0, 4.0 and 5.0 g kg^{-1} level of lyophilized stress-resistant yeast culture (Strain OBV9) and incubated with rumen fluid in 50 mL serum bottles under strict anaerobic conditions for 48 h as per the two stage technique of Tilley and Terry (Goering and Van Soest, 1970) to determine the optimum level of inclusion of yeast culture on *in vitro* digestibility.

The other *in vitro* study was performed as per the method (Menke *et al*., 1979) to evaluate the effect of stress-resistant yeast culture on the total *in vitro* gas production, *in vitro* organic matter digestibility and Metabolizable Energy (ME). The Truly Degraded Organic Matter (TDOM) and Microbial Biomass Production (MBP) were calculated using equations of Blummel *et al*. (1997). The mass of material solubilized from the pellet obtained following centrifugation of syringe contents after 24 h fermentation (amount of substrate truly degraded-amount apparently degraded) was calculated and used as an estimate of microbial biomass.

***In vivo* animal experiment:** A 180 day lactation trial was conducted to assess the effect of total mixed rations with and without the supplementation of both mesophilic (MTCC-1813) and stress-resistant (OBV-9) strains of yeast *Saccharomyces cerevisiae* on nutrient digestibility, milk yield and milk composition of buffaloes. Eighteen multi-parous, lactating, Murrah breed of buffaloes with an average body weight of 498.81±23.71 kg were selected from the Dairy Experimental Station (DES), Rajendranagar. These animals were randomly assigned to three treatments (1) Diet 1 (total mixed ration without yeast supplementation), (2) Diet 2 (total mixed ration with 1.0 g kg^{-1} level of mesophilic yeast culture (MTCC-1813) and (3) Diet 3 (total mixed ration with 1.0 g kg^{-1} level of stress-resistant yeast (OBV-9). The ingredient and chemical compositions of experimental feeds are as furnished in Table 2. All the animals were de-wormed, vaccinated before the onset of the experiment. Animals had access to clean drinking water and mineral block through-out the experimental period.

Samples for digestibility estimations were taken towards the end of the experiment for 15 days to assess the nutrient utilization.

Table 2: Composition of the total mixed rations with and without the supplementation of yeast culture

Ingredients (g kg^{-1})	Diet 1	Diet 2	Diet 3
Sorghum straw	400.0	400.0	400.0
Maize	140.0	140.0	140.0
Groundnut cake	125.0	125.0	125.0
Sunflower cake	150.0	150.0	150.0
Deoiled rice bran	100.0	100.0	100.0
Molasses	70.0	70.0	70.0
Mineral mixture	10.0	10.0	10.0
Salt	5.0	5.0	5.0
Yeast culture MTCC1813	-	1.0	-
Yeast culture OBV9	-	-	1.0
Chemical composition (g kg^{-1})			
Dry matter	886.5	886.5	886.5
Organic matter	889.8	889.8	889.8
Crude protein	118.8	118.8	118.8
Ether extract	19.0	19.0	19.0
Total ash	110.2	110.2	110.2
Cell wall constituents (proportion of crude fiber)			
Neutral detergent fibre	0.52	0.52	0.52
Acid detergent fibre	0.33	0.33	0.33
Hemicellulose	0.19	0.19	0.19
Cellulose	0.25	0.25	0.25
Lignin	0.05	0.05	0.05
Nutritive value			
DCP (g kg^{-1})	79.40	82.60	83.30
ME (MJ kg^{-1} DM)	8.78	9.20	9.33

DCP: Digestible crude protein, ME: Metabolizable energy. Vitamin AD_3 is added at 10 g/100 kg, Each value is an average of triplicate analysis

Collection of feed, feed residues and feces: Representative samples of each feed offered and leftovers were collected separately and pooled for 15 days to estimate proximate composition and cell wall constituents. The samples were ground separately in a laboratory Wiley mill through a 1 mm screen and preserved in air tight bottles for subsequent analysis. Feces from each animal was collected in separate containers weighed, mixed thoroughly and placed in a wide mouthed stopper bottles before taking to laboratory for analysis. For dry matter, aliquots of 1% of daily feces voided by each animal was taken in previously weighed petri dishes and dried overnight in hot air oven at 100±5°C. The daily sample from each animal for seven day collection period was pooled, ground in Wiley mill through a 1 mm screen and stored in polythene bags for further analysis.

For fecal nitrogen estimation, 0.1% feces voided each day by individual animal was weighed and frozen in refrigerator for further analysis.

Chemical analysis: Samples of experimental feed, leftover and feces were collected and analyzed for N using 'Turbotherm' and 'Vapodest' (Gerhard, Germany) analyzer based on the principle of Micro-Kjeldahl method (AOAC., 1997; procedure No. 4.2.02). DM, total ash and EE were determined according to the procedures (No. 4.1.03, 4.1.10, 4.5.01 and 4.6.01) described by AOAC (1997). The NDF and ADF of feeds and faeces were analyzed as outlined by Van Soest *et al.* (1991). Hemicellulose was calculated as NDF-ADF. ME values were calculated based on NRC (1978) formulae (1 kg TDN = 17.45 MJ DE; ME = DE×0.82).

Sampling of milk and milk analysis: Representative samples of milk from individual buffaloes were collected into sterile milk sample bottles after each milking (morning and evening) at monthly intervals to estimate fat, Solids Not Fat (SNF), total solids, protein and lactose yields per kg milk.

The milk fat was determined by Gerber's method (ISI., 1977) using special butyrometre and pipette with ISI marking. The Solids Not Fat (SNF) content was calculated by using the ISI formula based on estimation of specific gravity using corrected lactometer reading (BIS, 1982).

Total Solids (TS) of milk was estimated by using the ISI formula for TS percent:

$$TS = \frac{CLR}{4} + 1.22\ F + 0.72$$

where, CLR is corrected lactometer reading and F is dat percent.

Milk protein content was estimated based on the principle of Micro-Kjeldahl method (AOAC., 1997; procedure No. 4.2.02). Lactose was estimated based on the spectrophotometer method, where the sedimentation of balance substances in milk samples was achieved using sixty-five percent ethanol with acetic acid and the absorbance was measured at 410 nm wavelength. The lactose content was calculated according to the standard curve Petrushevska-Tozi and Bauer-Petrovska (1997). Somatic cell count in the milk was calculated according to the method described by Schalm *et al.* (1971). The dried smears of freshly collected milk samples were made on clean grease free glass slide and stained by "Newman Lampart stain". A total of twenty five randomly selected fields of microscope were counted under oil immersion and average microscopic factor for somatic cell count per milliliter of milk was calculated.

Statistical analysis: Data was analyzed statistically (Snedecor and Cochran, 1980) and tested for significance by Duncan's multiple range test (Duncan, 1955). Milk composition and milk constituents between different treatments were analyzed as per Mixed procedure of SAS (2011). Significant differences were accepted if $p \leq 0.05$.

RESULTS

This study encapsulates both *in vitro* and *in vivo* experiments for evaluation of the effects of supplementing stress-resistant yeast culture on lactating buffaloes.

***In vitro* assay:** Different levels (0.0, 1.0, 2.0, 3.0, 4.0 and 5.0) of stress-resistant yeast culture were evaluated for IVDMD and gas production using Tilley and Terry method and Menke's method (Table 3).

Table 3: Evaluation of different levels of stress-resistant yeast OBV9 on the *in vitro* digestibility and *in vitro* gas production using different methods

	Levels of yeast (g kg^{-1})						
Parameters	0.0	1.0	2.0	3.0	4.0	5.0	SEM
Tilley and terry *in vitro* digestibility							
IVDMD (Fractions)	0.503	0.538	0.541	0.533	0.539	0.541	0.01
ME (MJ kg^{-1} DM)	8.49	8.64	8.65	8.65	8.58	8.68	0.18
Menke's *in vitro* gas production							
Gas volume (mL)	41.3	42.37	42.43	42.45	41.9	42.32	1.32
IVOMD (mg)	59.36	60.26	60.31	60.33	59.86	60.22	1.12
TDOM* (mg)	115.10^{a}	132.11^{b}	129.48^{b}	128.3^{b}	129.33^{b}	126.95^{ab}	4.19
MBP* (mg)	55.74^{a}	71.85^{b}	69.17^{b}	67.97^{ab}	69.47^{b}	66.73^{ab}	4.35

IVDMD: *In vitro* dry matter digestibility, IVOMD: *In vitro* organic mater digestibility, TDOML: Truly digestible organic matter, MBP: Microbial biomass production, ME: Metabolizable energy, Each value is an average of triplicate analysis, *Calculated by using the formula of Blummel *et al.* (1997). ^{a,b,ab}Values with different superscripts in a row differ significantly ($p<0.05$)

Table 4: Effect of total mixed rations with and without supplementation of yeast cultures on nutrient digestibility and intake in lactating buffaloes

Nutrient digestibility	Diet 1	Diet 2	Diet 3	SEM
n	6	6	6	
Dry matter	0.53[a]	0.55[ab]	0.58[b]	0.02
Crude protein	0.67	0.69	0.70	0.02
Ether extract	0.71	0.72	0.74	0.02
Neutral detergent fibre	0.53	0.55	0.56	0.02
Acid detergent fibre	0.45	0.49	0.52	0.01
Hemicellulose	0.65[a]	0.65[a]	0.71[b]	0.01
Cellulose	0.58[a]	0.60[b]	0.64[c]	0.01
Intake				
DCP (g day^{-1})	1001.10	1031.10	1017.00	47.90
ME (MJ day^{-1})	110.40	115.30	120.60	6.17

DCP: Digestible crude protein, ME: Metabolizable energy, [abc]Values bearing different superscripts in a row differ significantly ($p<0.05$)

Tilley and terry *in vitro* digestibility: The IVDMD coefficients of yeast supplemented groups were numerically higher ($p>0.05$) than controls. The ME value followed the same pattern.

Menke's method: The IVOMD and total gas production were not affected by yeast supplementation. However, true degradable organic matter (TDOM as mg 200 mg^{-1} DM) and microbial biomass production (MBP as mg 200 mg^{-1} DM) values were significantly ($p<0.05$) higher for yeast (1.0, 2.0 and 4.0 g kg^{-1} diet level) supplemented groups over control group. For 3.0 g kg^{-1} yeast supplemented group, the MBP values were significantly ($p<0.05$) higher compared to control ration. There was no difference between different levels of yeast supplementation on any of the variables tested.

***In vivo* experiment:** An animal experiment was conducted to evaluate the effect of stress-resistant yeast culture (Diet 3) on the nutrient digestibility and milk production performance of lactating buffaloes against a control (Diet 1, no yeast supplementation) and a positive control which is fed with mesophilic yeast culture (Diet 2).

Influence of yeast supplementation on nutrient digestibility: Diet 3 increased the DM digestibility ($p<0.05$) compared to Diet 2 and Diet 1 (Table 4). While the NDF digestibility did not seem to be influenced with yeast supplementation, individual constituents of fibre, the digestibilities of cellulose and hemicellulose were significantly ($p<0.05$) improved with stress-resistant yeast.

Nutritive value and nutrient intake: The ME and DCP intakes of both supplemented groups (Diet 2 and 3) were more than the recommended levels of ICAR (1998).

Milk yield and composition: The average milk yield (kg day^{-1}) and daily average feed intake kg^{-1} milk yield of Diet 3 were numerically ($p>0.05$) lower than the Diet 2 and Diet 1 (Table 5).

The total solids and Solids Not Fat (SNF) of milk in lactating murrah buffaloes was not significantly ($p>0.05$) influenced by the Diet 3 compared to the Diet 2 and Diet 1 (Table 5). The daily average butter fat and lactose yield as g kg^{-1} milk of the lactating Murrah buffaloes tended to be higher for Diet 3 ($p<0.07$). The milk protein (g kg^{-1} milk) was higher in Diet 3 ($p<0.05$) compared to Diet 1 and Diet 2. The somatic cell count was lower ($p<0.05$) for Diet 3 compared to the other two diets.

Table 5: Effect of total mixed ration with and without the supplementation of yeast culture on the milk yield, dry matter intake and milk composition in lactating buffaloes

	Total mixed ration			
Parameters	Diet 1	Diet 2	Diet 3	SEM
Milk yield (kg day^{-1})	6.39	6.62	6.75	0.59
Feed intake (kg)/kg milk yield	2.78	2.61	2.50	0.29
DMI (kg day^{-1})	12.56	12.52	12.20	0.49
Milk components (g kg^{-1})				
Total solids	178.55	178.94	180.18	1.30
SNF[1]	103.11	103.81	104.25	1.12
Fat[†]	70.52	70.92	72.63	0.80
Protein	38.26^{a}	39.06^{ab}	41.81^{b}	1.10
SCC[2] (count mL)	76061.00^{a}	69038.00^{b}	65007.00^{c}	45.27
Lactose[†]	34.43	34.88	36.63	0.80

[1]SNF: Solid not fat, [2]SCC: Somatic cell count, DMI: Dry matter intake, Each value is an average of six observations, ^{abc}Values bearing different superscripts in a row differ significantly (p<0.05), †Diet 3 is tended to be significant (p<0.06) compared to diet 1

DISCUSSION

We isolated a strain of *Saccharomyces cerevisiae* (OBV-9) from brewery effluents that has the ability to survive at higher temperatures as in the rumen and henceforth we described the isolate as stress-resistant yeast culture (Bhima *et al*., 2010). The strain OBV-9 has the potential to be used as a probiotic in animal feeds which was evaluated in this study through a combination of *in vitro* and *in vivo* experiments.

The *in vitro* study summarized that the yeast strain (OBV-9) at a concentration of 1 g kg^{-1} DM showed the best results based on *in vitro* digestibility and microbial biomass production. In the past, *in vitro* fermentation and microbial biomass production assays were used to evaluate the efficacy of alternate feed supplements (Harikrishna *et al*., 2012). *In vitro* experiments using yeast supplementation when used at similar concentrations to our study were not able to improve microbial biomass production (Harrison *et al*., 1988; Agarwal *et al*., 2000). Therefore, stress-resistant yeast strain (OBV-9) appears to be more effective compared to other yeast strains.. From our *in vitro* assays, it could be inferred that our yeast culture strain at 1 g kg^{-1} DM possibly promoted the growth and multiplication of microbial populations which led to the increase in total microbial biomass production which ultimately favored both *in vitro* digestibilities and also gas production.

From our *in vivo* studies, we found an increase in the DM digestibility with yeast supplementation similar to the reports of Haddad and Goussous (2005), who supplemented yeast along with forage based diets fed to fattening lambs. Our findings were unable to detect an effect of yeast supplementation on either protein or fiber digestibility contrary to the findings as reported (Wohlt *et al*., 1991; Rao *et al*., 1997; Saha *et al*., 1999; Elseed *et al*., 2007). However, we found that yeast supplementation increased digestibilities of both cellulose and hemicellulose similar to that of Fallon (1987). Improved fibre digestibility (Beauchemin *et al*., 2006) and increases in cellulose and hemicellulose digestibility due to stress-resistant yeast supplementation as observed in this study is probably due to an increased bacterial growth as yeast culture provides necessary growth promoters, vitamins required for their growth (Chaucheyras-Durand *et al*., 2008).

The calculated ME value in the supplemented treatments was comparable with findings of yeast supplementation to Holstein cows (Dolezal *et al*., 2005). There was no effect of yeast supplementation on the average milk yield of buffaloes which was similar to several reports (Besong *et al*., 1996; Nursoy and Baytok, 2003). However, yeast supplementation had an influence on the milk constituents such as protein, lactose and butter fat similar to previous reports (Garg *et al*., 2000; Yalcin *et al*., 2011).

Future studies should be directed towards testing the efficacy of the stress-resistant yeast culture (OBV9) on the rumen microbiome as yeast supplementation can influence the growth of microbial populations and stabilize pH in the rumen (Newbold *et al*., 1998; Williams *et al*., 1991; Yoon and Stern, 1996). Also, the effect of stress-resistant yeast on fiber digestion needs to be evaluated as yeast supplementation favored the growth of *Ruminococcus*, *Eubacterium, Selenomonas, Butyrivibrio* and certain genera of the *Lachnospiraceae* and *Clostridaceae* which are associated with fiber digestion in the rumen (Teather and Wood, 1982).

CONCLUSION

The results of the present study indicated that the thermo, acid, osmo and bile tolerant probiotic yeast *Saccharomyces cerevisiae* OBV9 based complete diet (Diet 3) showed positive tendency in improving digestibilities of dry matter, crude fiber, cellulose, hemicellulose, milk proteins, lactose, butter fat and somatic cell count in milk. It also showed slightly positive tendency in improving milk yield, solids not fat yield. However, these improvements were not statistically significant compared to other diets. Therefore, it is concluded that the stress-tolerant yeast supplementation to lactating Murrah buffalos was beneficial to some of the nutrient digestibilities and milk components but did not significantly influence the milk production.

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