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Quality evaluation of pearl millet silages using *Saccharomyces cerevisiae* as silage additive

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ABSTRACT

The present study was conducted to ascertain the quality of pearl millet-sorghum and pearl millet-cowpea fodders (PM-S and PM-C) with their respective silages made with or without *S. cerevisiae* as silage inoculant in polybags. On opening the bags after 45 days all the silages were free from moulds and had comparable pH. The WSC was highest in fodders and reduced significantly after ensiling. Use of inoculant improved the CP in PM-C silage and increased the *td*CPf, TDN_{1X}, DE and ME of both mixed fodder silages. The NDFIN, ADFIN and ADFIN as percentage of total N were reduced in the *S. cerevisiae* inoculated silages. The NH_3–N was higher in PM-S silage inoculated with *S. cerevisiae* but the pH and NH_3-N as percentage of total N remained within the acceptable limits reflecting no adverse effect of the inoculant. Use of *S. cerevisiae* in PM-S and PM-C during ensiling reduced the plant protein breakdown and fiber bound nitrogen, increased the truly digestible protein and energy availability and improved aerobic stability through better buffering capacity and acetic acid content. It was concluded that *Saccharomyces cerevisiae* as inoculant during ensiling of pearl millet-sorghum and pearl millet-cowpea improved the fermentative process of the silages prepared in polybags.

Key words: Digestibility, Pearl millet-sorghum, Pearl millet-cowpea, *Saccharomyces cerevisiae,* Silage quality

Maize the preferred summer fodder crop has the disadvantage of being very vulnerable to water stress and cannot regrow. Punjab has been experiencing water crises and dry water conditions close to drought during summer. Dairy production continues to grow with an increasing gap between demand and supply of feed. The gap can be bridged by growing summer annual crops like pearl millet and sorghum as an alternative forage crop. The hybrids of these crops have the potential to compete with maize silage in terms of yield and nutritive value. The disadvantage of feeding these crops is the potential to cyanide and nitrate poisoning. However, during ensiling the HCN and nitrate reduces.

Use of mixed fodder of pearl millet and sorghum/cowpea can increases the energy and/or protein supply to the animals. During ensiling anerobic conditions reduce pH and stimulate growth of lactic acid bacteria (Chen and Weinberg 2009). Quality of silage could be improved with *Saccharomyces cerevisiae* which stimulates growth of cellulolytic and lactic acid utilising bacteria. Recent research indicated that *S. cerevisiae* produce an active compound oxylipin that plays a role in clostridia inhibition (Strauss *et al.* 2005). Sofyan *et al.* (2010) in laboratory study reported that lactobacillus alone or in combination with *Saccharomyces cerevisiae* could be used as silage additive. Hence, this study was planned to evaluate the quality of silage made from pearl millet-sorghum and pearl millet-cowpea fodders with and without *Saccharomyces cerevisiae* as silage additive in polybags.

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MATERIALS AND METHODS

Preparation of silage inoculants: The *Saccharomyces cerevisiae* culture was inoculated @ 2% at 37°C for 24 h in a mixture of 2 kg cracked maize grain in 4 litre water. Fermented maize grain contained live microbes @ 2×10^8 cell/g. Lyophilized *Saccharomyces cerevisiae,* strain NCDC-49 was procured from IVRI, Izatnagar and was primarily cultured in glucose yeast extract broth (Chaudhary *et al*. 2002). Primary culture of *S. cerevisiae* was standardized by haemocytometer method using Neubar's counting chamber. Numbers of CFUS were calculated by using formula (Sharma 2007).

Preparation of silage: Three quintals of unwilted (DM 25%) chaffed (3–5 cm length) fodders of pearl millet-sorghum (PM-S) and pearl millet-cowpea (PM-C) were preserved as silages in polybags made of polyethelene of 50 micron thickness, 6 feet length and 3 feet diameter (Saijpaul *et al*. 2013), without *Saccharomyces cerevisiae*

(PM-SC and PM-CC) as control or with *Saccharomyces cerevisiae* (5% of fresh fodder) as silage inoculant in treatment groups PM-ST and PM-CT, respectively.

Analytical procedures: The water extract of the fodders and silages (10g/100ml) was analyzed for pH using a digital pH meter, individual and total VFA (Coltyn and Boucque 1968) using gas chromatography equipped with flame ionization detector. The total nitrogen (TN) and NH_3-N (AOAC 2000), buffering capacity (Plyne and McDonald 1966) and water soluble carbohydrates (WSC) as per Dubois *et al*. (1956) of the extract were estimated. The dry ground samples of fodders and silages were analyzed for CP, ash and ether extract (AOAC 2000), cell-wall fractions and nitrogen bound acid-detergent fibre (ADIN) and neutral detergent fiber (NDFIN) as per Goering and Van Soest (1970). The non-fiber carbohydrates (NFC) was calculated by difference and truly digestible NDF (*td*NDF) truly digestible non-fiber carbohydrates (*td*NFC), truly digestible crude protein fodder (*td*CPf), percent total digestible nutrients at maintenance (TDN_{1X}), DE, ME and NE_L (Mcal/kg) were calculated using equations (NRC 2001).

Statistical analysis: Data were analyzed by one way ANOVA to test the difference between pearl millet-sorghum and pearl millet-cowpea fodder and silages (Snedecor and Cochran 1994). The significant means between different treatments were compared by Tukey's b-test.

RESULTS AND DISCUSSION

The polybags were opened on day 45 and silages were found well conserved with no visible signs of mould growth. The chemical composition of pearl millet-sorghum (PM-S) and pearl millet-cowpea (PM-C) fodders and their silages without (PM-SC and PM-CC) and with *S. cerevisiae* (PM-ST and PM-CT) are given in Table 1. Fermentation during silage making reduced the OM and EE in PM-S. In PM-C the OM remained similar but EE increased as compared to the fodder. The CP in PM-S silages remained comparable to the fodder but decreased (P<0.05) in PM-C silages. The WSC was highest in the fodders and reduced significantly after ensiling due to conversion to organic acids. In both

Table 1. Chemical composition (% DM) of pearl millet-sorghum and pearl millet-cowpea fodders and silages with and without *S. cerevisiae*

Parameters	PM-S	PM-SC	PM-ST	SEM	PM-C	PM-CC	PM-CT	SEM
OM	91.77 ^{a}	89.18 ^{b}	90.62 ^{b}	0.49	89.60	89.96	89.36	0.14
CP	7.50	7.00	7.25	0.09	8.50^{y}	8.00^{x}	8.2 0^{x}	0.09
EE	2.50^{a}	1.47^{b}	1.87^{b}	0.19	1.05^{x}	2.55^{y}	2.67 ^{y}	0.33
WSC	11.94 ^{a}	0.90^{b}	0.67^{b}	2.35	9.50 ^{x}	0.63 ^{y}	0.67 ^{y}	1.86
Total carbohydrates	81.77	80.71	81.49	0.23	80.05	79.41	78.50	0.30
NFC	14.37^{b}	8.21^{a}	9.59^{a}	1.22	15.55	15.01	15.50	0.20
td NFC	46.49^{b}	42.25^{a}	41.24^{a}	1.03	54.51	55.68	56.70	0.45
tdCPf	6.90^{c}	4.85^{a}	6.08^{b}	0.38	7.79 ^{x}	6.41 ^{y}	7.41 ^{x}	0.26
TDN_{1x}	89.31^{c}	83.48^{a}	84.74^{b}	1.12	86.65^{x}	91.27^{y}	93.04^{z}	1.20
DE (Mcal/kg)	3.94^{c}	3.68^{a}	3.74^{b}	0.05	3.82^{x}	4.02^{y}	4.10^{z}	0.05
ME (Mcal/kg)	3.53^{c}	3.27^{a}	3.32^{b}	0.05	3.41^{x}	3.61^{y}	3.69^{z}	0.05
NE_L(Mcal/kg)	2.19	2.04	2.08	0.04	2.12^{x}	2.24^{y}	2.28^{y}	0.03

*Means bearing different superscripts a,b,c and x,y,z within a row differ significantly (P < 0.05). PM-S, Pearl millet-sorghum fodder; PM-SC, pearl millet-sorghum silage control; PM-ST, pearl millet-sorghum silage with *S. cerevisiae* as inoculants; PM-C, pearl millet-cowpea fodder; PM-CC, pearl millet-cowpea silage control; PM-CT, pearl millet-cowpea silage with *S. cerevisiae* as inoculant.

Table 2. CNCPS fractions (% DM) of pearl millet-sorghum and pearl millet-cowpea fodders and silages with and without *S. cerevisiae*

Parameters	PM-S	PM-SC	PM-ST	SEM	PM-C	PM-CC	PM-CT	SEM
NDF	67.40^{a}	72.50^{b}	71.40^{b}	1.01	64.50	64.40	63.00	0.40
ADF	39.00^{a}	47.90^{b}	48.20^{b}	2.01	41.5	44.10	43.60	0.68
Hemicellulose	28.40	24.60	23.20	1.22	23.00	20.30	19.40	0.88
Cellulose	33.20	39.20	39.70	1.42	31.00^{x}	35.40^{y}	33.70^{xy}	0.85
ADL	3.60	4.70	5.90	0.49	5.20	5.70	5.60	0.58
NDFIN	0.39^{a}	0.30 ^{b}	0.19^{c}	0.04	0.38^{z}	0.294^{y}	0.20^{x}	0.03
ADFIN	0.08^{a}	0.34^{b}	0.19^{a}	0.05	0.10^{x}	0.24^{y}	0.11^{x}	0.28
ADFIN (% total N)	14.90^{a}	54.92^{b}	30.39^{a}	7.52	11.83 ^{x}	37.91^{y}	13.25^{x}	5.50
td NDF	39.34	41.33	38.99	0.50	34.53	34.02	33.62	0.20

*Means bearing different superscripts a,b,c and x,y,z within a row differ significantly (P < 0.05). PM-S, Pearl millet-sorghum fodder; PM-SC, pearl millet-sorghum silage control; PM-ST, pearl millet-sorghum silage with *S. cerevisiae* as inoculants; PM-C, pearl millet-cowpea fodder; PM-CC, pearl millet-cowpea silage control; PM-CT, pearl millet-cowpea silage with *S. cerevisiae* as inoculant.

the fodders and their silages the total carbohydrates remained unchanged but the NFC reduced in PM-S silages only. The *td*NFC and *td*CPf were higher in PM-S fodder than its silages but use of *S. cerevisiae* increased the *td*CPf against control (PM-SC). The *td*CPf was similar in PM-C fodder and PM-CT silage but higher (P<0.05) than PM-CC silage. The TDN_{1X} and DE were higher in PM-S fodder than its silages but use of *S. cerevisiae* increased the TDN_{1X} and DE against control (PM-SC). The TDN_{1X}, DE, ME and NE_L of PM-C fodder was lower (P<0.05) than its silages. Highest TDN_{1X}, DE and ME were observed in the *S. cerevisiae* inoculated silage of PM-C.

The CNCPS fractions of pearl millet-sorghum and pearl millet-cowpea fodder and silages with and without *S. cerevisiae* (Table 2) indicated that compared to fodder the silage making process increased the NDF and ADF in PM-S but not in PM-C. The hemicellulose and ADL of both the fodders and their silages remained comparable. Cellulose fraction increased in the PM-CC silage. Ensiling reduced the NDFIN in both the crops as compared to the fodders but use of *S. cerevisiae* further reduced (P<0.05) the NDFIN. The ADFIN of the fodder and *S. cerevisiae* inoculated silages remained similar but increased in the control silages of both PM-S and PM-C. The ADFIN as per cent of total N was higher in PM-SC and PM-CC silages but use of inoculant reduced (P<0.05) this fraction to the level of their respective fodders. The *td*NDF of the fodders and their silages remained unchanged.

The silage quality parameters given in Table 3 showed that the pH of the water extract of both PM-S and PM-C fodders was higher as compared to their silages which reduced (P<0.05) due to the fermentative process. The pH of PM-SC and PM-ST remained comparable which was in accordance with Kamali *et al.* (2012). However, the pH of the *S. cerevisiae* inoculated PM-CT silage was significantly lower than its control silage. The result indicated that when a non-legume fodder is ensiled the sugars bring down the pH during fermentation. However, when a legume fodder is added during ensiling the higher protein content act as a buffer and reduces the chances of pH reduction during ensiling. On addition of *S. cerevisiae* as inoculant, the pH reduced further may be due to more growth of cellulolytic and lactic acid utilizing bacteria as reported earlier (Chaucheyras *et al.* 1995). Bacterial inoculants tend to increase the rate and extent of lactic acid fermentation which lower the pH of silage. The use of mixed fodder of pearl millet and cowpea or its silage can increase the nitrogen supply to ruminants. Ammonia nitrogen is an indicator of peptides and amino acid degradation by clostridium microorganisms. The NH_3-N was lowest in the fodders. Preservation of PM-S and PM-C fodders as silage increased the NH_3-N being highest in PM-ST against its control (PM-SC) but their NH_3-N % of total N remained similar. In PM-C silages the NH_3-N remained similar so, the NH_3-N % of total N of the fodder and silages were comparable. Marked difference was observed in the buffering capacity which was highest in the inoculant based PM-S silage followed by control silage and it was lowest in the fodder water extract. Buffering capacity (BC) in silage can be defined as the degree to which forage material resists change in pH. Silage with higher BC value could exhibit greater aerobic stability. Results indicated that presence of *S. cerevisiae* improved the BC in PM-S silage and this may be useful in maintaining the quality of silage during feedlot. In PM-C ensiling increased the buffering capacity of the silages irrespective of the presence of additive. Acetic acid production begins in the second phase of silage fermentation by acetic acid bacteria. The acetic acid and butyric acid were significantly higher in PM-ST compared to PM-SC silage resulting in highest TVFA in the silage with *S. cerevisiae* as inoculants. In PM-C acetic acid was higher and butyric acid lower (P<0.05) in the silage inoculated with *S. cerevisiae* when compared to the control (PM-CC) indicating better anaerobic fermentability through cellulolytic and lactic acid utilizing bacteria but TVFA remained comparable.

The comparative evaluation of pearl millet-sorghum and pearl millet-cowpea silages with and without S. *cerevisiae* (Table 4) clearly showed that use of inoculant improved the CP in PM-CT silage reflecting more protein availability

Table 3. Silage quality parameters of pearl millet-sorghum and pearl millet-cowpea fodders and silages with and without *S.cerevisiae*

Parameters	PM-S	PM-SC	PM-ST	SEM	PM-C	PM-CC	PM-CT	SEM
pH	5.58 ^{a}	4.22 ^{b}	4.29^{b}	0.28	6.30 ^{z}	4.82 ^{y}	4.55 ^{x}	0.34
NH_3- N(mg/100g DM)	24.84^{a}	47.04^{b}	53.76^{c}	5.38	58.24^{x}	150.08^{y}	129.92^{y}	7.48
NH_3- N as % of total N	2.07 ^{a}	4.20^{b}	4.63^{b}	1.31	4.28	11.72	9.90	0.37
Buffering capacity (mM/100g DM)	30.62^{a}	62.50^{b}	94.85^{c}	11.74	27.44 ^{x}	67.80^{y}	78.44 ^{y}	10.13
Volatile Fatty acids (mM/100g DM)								
Acetic acid	21.55^{a}	29.87^{a}	90.73^{b}	13.90	18.65^{x}	33.44^{x}	67.93^{y}	9.41
Propionic acid	0.00^{a}	0.87^{ab}	2.57^{b}	0.50	4.19	6.14	3.55	0.54
Butyric acid	0.00^{a}	1.70^{a}	11.37^{b}	2.31	0.00^{x}	44.81^{z}	19.04^{y}	8.24
Total VFA	21.55^{a}	32.44^{a}	104.67^{b}	16.61	22.83^{x}	86.27^{y}	90.52^{y}	14.08

*Means bearing different superscripts a,b,c and x,y,z within a row differ significantly (P < 0.05). PM-S, Pearl millet-sorghum fodder; PM-SC, pearl millet-sorghum silage control; PM-ST, pearl millet-sorghum silage with *S. cerevisiae* as inoculants; PM-C, pearl millet-cowpea fodder; PM-CC, pearl millet-cowpea silage control; PM-CT, pearl millet-cowpea silage with *S. cerevisiae* as inoculant.

Table 4. Nutritive value and silage quality of pearl millet-sorghum and pearl millet-cowpea silages with and without *S. cerevisiae*

Parameters	PM-SC	PM-ST	PM-CC	PM-CT
Nutritive value (% DM)				
OM	89.18	90.62	89.96	89.36
CP	7.00	7.25	8.00^{x}	8.2 0^{y}
EE	1.47	1.87	2.55	2.67
WSC	0.90	0.67	0.63	0.67
Total carbohydrates	80.71	81.49	79.41	78.50
NFC	8.21	9.59	15.01	15.50
td NFC	42.25	41.24	55.68	56.70
tdCPf	4.85^{a}	6.08^{b}	6.41^{x}	7.41^{y}
TDN_{1x}	83.48^{a}	84.74^{b}	91.27^{x}	93.04^{y}
DE (Mcal/kg)	3.68^{a}	3.74^{b}	4.02^{x}	4.10^{y}
ME (Mcal/kg)	3.27^{a}	3.32^{b}	3.61^{x}	3.69^{y}
NE_L(Mcal/kg)	2.04	2.08	2.24	2.28
Cell wall fractions (%)				
NDF	72.50	71.40	64.40	63.00
ADF	47.90	48.20	44.10	43.60
ADFIN	0.34^{b}	0.19^{a}	0.24^{y}	0.11^{x}
NDFIN	0.30 ^{a}	0.19^{b}	0.294^{y}	0.20^{x}
ADFIN as % of total N	54.92^{b}	30.39^{a}	37.91^{y}	13.25^{x}
Hemicellulose	24.60	23.20	20.30	19.40
Cellulose	39.20	39.70	35.40	33.70
ADL	4.70	5.90	5.70	5.60
td NDF	41.33	38.99	34.02	33.62
Silage quality (mM)/100g DM				
pH	4.22	4.29	4.82^{y}	4.55^{x}
Ammonical N (mg/100g DM)	47.04^{a}	53.76^{b}	150.08	129.92
Amm. N as % of total N	4.20	4.63	11.72	9.90
Buffering capacity	62.50^{a}	94.85^{b}	67.80	78.44
Acetic acid	29.87^{a}	90.73^{b}	33.44^{x}	67.93^{y}
Propionic acid	0.87	2.57	6.14	3.55
Butyric acid	1.70^{a}	11.37^{b}	44.81^{y}	19.04^{x}
Total VFA	32.44^{a}	104.67^{b}	86.27	90.52

*Means bearing different superscripts within a row differ significantly ($P < 0.05$). PM-S, Pearl millet-sorghum fodder; PM-SC, pearl millet-sorghum silage control; PM-ST, pearl millet-sorghum silage with *S. cerevisiae* as inoculants; PM-C, pearl millet-cowpea fodder; PM-CC, pearl millet-cowpea silage control; PM-CT, pearl millet-cowpea silage with *S. cerevisiae* as inoculant.

to the animals. Additionally, the increased *td*CPf, TDN_{1X}, DE and ME in the inoculated silages of both the mixed fodders meant more protein and energy would be digested from these silages. The lower NDFIN, ADFIN and ADFIN as % of total N in the *S. cerevisiae* inoculated silages indicates less fiber bound N or release of fiber bound N by S. *cerevisiae* which could have resulted in higher digestible N (*td*CPf). The higher NH_3-N in PM-S silage inoculated with *S. cerevisiae* could be due to reduction in fiber bound N but the pH and NH_3-N % of total N remained within the acceptable limits reflecting no adverse effect of the inoculant. The higher acetic acid may be due to the stimulus provided by cellulytic and lactic acid utilizing bacteria as addition of *S. cerevisiae* live cells to cultures of some cellulolytic fungal species has shown to stimulate zoospores germination and cellulose degradation.

Based on these findings it can be summarized that use of *Saccharomyces cerevisiae* as inoculant in pearl millet-sorghum and pearl millet-cowpea silages reduced the plant protein breakdown and fiber bound nitrogen, increased the truly digestible protein and energy availability and improved aerobic stability through better buffering capacity and acetic acid content. Hence, it can be used as silage additive to improve the fermentative process and establishing aerobic stability to the pearl millet-sorghum and pearl millet-cowpea silage prepared in polybags.

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