Effects of yeast (*Saccharomyces cerevisiae*) supplementation on intake, digestibility, rumen fermentation and milk yield in Nili-Ravi buffaloes

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Summary

Rumen Yeast® (RY; *Saccharomyces cerevisiae*), a live yeast strain, improves milk yield and composition and nutrients digestibility through balancing rumen ecosystem and increasing ruminal cellulolytic bacteria numbers in cattle. To examine the effects of dietary supplementation of RY in Nili-Ravi buffaloes, 16 buffaloes with 8 L average daily milk production were randomly divided into two groups, and investigated for a 60-day period. Group I (control) was offered maize silage *ad libitum* as sole forage plus 3 kg of concentrate/head per day (16% crude protein (CP) and 72% total digestible nutrients (TDN)), while group II was given the same diet as control supplemented with RY (14 g/head per day). Feed intake, nutrient digestibility, rumen fermentation and milk production of each animal were recorded. Average dry matter (DM) intake was not affected (P>0.05) in buffaloes with or without RY (14.7 and 14.3 kg/day, respectively). Digestibility of DM, CP, and ruminal pH were similar (P>0.05) between the groups, but the digestibility coefficients of neutral detergent fiber and acid detergent fiber were greater (P<0.05) for the animals that received RY. Milk production (9.60 vs. 9.15 L/day) and 4% fat corrected milk (FCM) (11.32 vs. 11.85 L/day) were significantly (P<0.05) greater in the buffaloes fed with RY than the control group. Milk composition was similar between the experimental groups, however, milk somatic cell count (SCC) was significantly (P<0.01) lower in RY supplemented buffaloes than the control animals. In conclusion, feeding RY had positive effects on milk production, fibre digestibility and SCC in buffaloes fed maize silage-concentrate based diet.

Key words: Digestibility, Intake, Lactating buffaloes, Milk production, Yeast (*Saccharomyces cerevisiae*)

Introduction

Buffalo has been the mainstay of the rural economy of small farmers in many of the developing countries of South Asia including Pakistan (Khan, 2009). Buffalo is normally kept as a dual-purpose animal i.e., both for milk and meat. Feeding cost contributes 60-70% of total animal production cost (Sindhu *et al*., 2002; Anjum and Afzal, 2015). Buffalo has better digestive ability than cattle to utilize poor quality roughages (Agarwal *et al*., 2008). Normally buffaloes are kept on green forages/roughages during maintenance, and offered concentrates (supplemented feeds) only during milking stage that may sometimes causes unstable rumen environment, poor digestion and absorption of costly nutrient and thus increases production cost (Sarwer *et al*., 2009). In this scenario, there is a dire need to introduce biotechnological tools and methods to stablize rumen ecosystem and enhance utilization efficiency of available feed resources (i.e., forages, crop residues and agro-industrial by-products) to reduce feeding cost of buffalo production.

Yeast supplementation in diets of ruminants is one option to increase utilization of poor quality roughages, grains and by-product based diets (Shriver-Munsch, 2011). Previous researchers (Moallem *et al*., 2009; Degirmencioglu *et al*., 2013; Meller *et al*., 2014) outlined some benefits of live yeast supplementation as increase in milk yield, milk protein, fibre digestion and stabilization of rumen pH in dairy cattle. The others reported that live yeast addition may balance rumen ecosystem and increase cellulolytic bacteria numbers in cattle (Wadhwa and Bakshi, 2013) and sheep (Mosoni *et al*., 2007). However, some researchers proposed that the effect of yeast supplementation is more pronounced in animals under stress conditions (Schingoethe *et al*., 2004; Moallem *et al*., 2009). Jouany and Morgavi (2007) reported that live yeast increases fibre digestion by stimulating cellulolytic bacteria and increases flow of microbial protein from the rumen. Furthermore, Bakr *et al*. (2015) noticed the decreased rumen ammonia nitrogen (N) concentration and increased ruminal pH, total volatile fatty acids (VFAs) and cellulose digestion in cattle that received the diet supplemented with yeast. Although good information on the effect of yeast supplementation is available in cattle, data on the effect of feeding live yeast in dairy buffaloes is scarce. Therefore, this study was planned to evaluate the effects of Rumen Yeast® (RY; S*accharomyces cerevisiae*) supplementation on nutrients digestibility, feed intake,

milk yield, milk composition, rumen fermentation parameters and economic viability in Nili-Ravi buffaloes fed maize silage-concentrate based total mixed ration.

Materials and Methods

Dairy buffaloes, feeding and management

Sixteen Nili-Ravi buffaloes, about 35 to 50 days in milk (2nd and 3rd production cycle), with average milk production of 8 ± 0.40 L/day were taken from Livestock Research Station, National Agricultural Research Centre (Islamabad, Pakistan). These buffaloes were kept in individual tie stalls. After acclimatization for 7 days, the animals were randomly allocated into two groups of 8 buffaloes each, and investigated for a 60-day period. Group I (control) was offered maize silage as sole forage *ad libitum* plus 3 kg of concentrates/head per day (16% CP and 72% TDN). Group II received the same diet as of control plus 14 g of RY/head. The RY consisted of 15 billion *S. cerevisiae* cells per g which released a considerable amount of metabolites (mannans and beta glucans) to improve microbial activity in the rumen. Concentrate was formulated as 25% rice polishing, 25% wheat bran, 15% maize gluten feed, 15% cane-molasses, 8% maize grains, 7% rapeseed cake, 3% cottonseed cake, 0.5% common salt, 0.5% di-calcium phosphate, 0.5% mineral premix and 0.5% urea, on a dry matter (DM) basis. This concentrate contained 90.20% DM, 16.08% CP, analyzed by method of AOAC (1990), and 33.59% neutral detergent fibre (NDF), 20.87% acid detergent fibre (ADF), determined by Van Soest *et al*. (1991), and 72.00% TDN. The TDN was calculated by regression equation based on proximate composition as described by Wardeh (1981). Maize silage was prepared mechanically in bales at Livestock Research Station during September which contained 33.00% DM, 8.90% CP, 48.50% NDF, 25.90% ADF (on a DM basis), and pH = 3.82. Feeding the animals was done twice daily at approximately 0830 and 1600 h. Total quantity of feed offered and refused by each animal was recorded daily to get feed intake. Fresh water was offered 3-4 times per day to all buffaloes. The buffaloes were milked twice a day, at approximately 0200 and 1400 h. Daily milk production was recorded and milk samples were collected fortnightly for chemical analyses. The trial lasted for 60 days (October to December, 2016) including the last 5 days for total collection of faeces for determination of nutrients digestibility. For that, feeds and orts samples (one sample/animal per day) were obtained, and composited by each buffalo for chemical analysis. Faeces were weighed daily and 10% of total faeces were composited by each buffalo, dried first at 60°C and then at 100°C in air forced oven, grinded, and analyzed for proximate composition according to AOAC (1990), and NDF and ADF by Van Soest *et al*. (1991). Hygienic environment was maintained by cleaning of floor with water. Milk fat, protein, total solids and somatic cell counts (SCC) were measured using MilkoScan analyser (FOSS ANA MilkoScan FT 120, GERBER INSTRUMENTS, Switzerland) according to the methods of Zecconi *et al*. (2002). Estimation of 4% fat corrected milk (FCM) was done by method described in NRC (2001) as:

$$4\%\ FCM = 0.4 \times \text{milk yield (kg/day)} + 15 \times \text{fat yield (kg/day)}$$

while solids non fat (SNF) were calculated as total solids minus fat.

During the last week, rumen fluid was collected from all buffaloes using a stomach tube, 3 h after morning feeding. Approximately 100 ml of rumen fluid was collected from each animal into a clean, dry flask as described by Shen *et al.* (2012) and ruminal pH was immediately measured using a portable digital pH meter (350-JANWAY, UK). Concentration of VFAs was assayed using gas chromatography (GC-Auto-system, Perkin Elmer, USA) according to the method of Hu *et al*. (2005). Rumen ammonia-N concentration was determined according to the method described by Conway (1974).

Economic benefit

The economic returns, expressed as the ratio of output to input, was calculated as:

$$\text{Output/input} = (MP \times MPM) / (DMI \times MPF)$$

Where,
MP: The average milk produced (L/head)
MPM: The average market price of milk ($/L)
DMI: The daily DM intake (DMI; kg/head per day)
MPF: The market price of feeds (Xie *et al*., 2012)

Statistical analysis

The data collected on different parameters were subjected to statistical analysis using t-test for means comparison between control group versus RY supplemented group at $P<0.05$ and $P<0.01$ levels of probability (Steel *et al*., 1997).

Results

Results of present study on body weight (BW) changes, DMI, milk production, FCM and milk composition (protein, fat, SNF, total solids and SCC) are given in Table 1. Rumen yeast supplementation had positive effect on the BW changes of the lactating buffaloes ($P<0.05$). Total DMI was 2.8% greater in buffaloes fed with RY compared to the control animals (14.7 and 14.3 kg, respectively), however, this increase was not statistically significant ($P>0.05$).

Average daily milk production and 4% FCM increased by 4.3% (9.60 vs. 9.15 L) and 4.7% (11.85 vs. 11.32 L) in RY supplemented buffaloes compared to control group, respectively ($P<0.05$). Milk protein, fat, SNF and total solids were similar ($P>0.05$) between the groups, whereas, SCC was lower ($P<0.01$) in the buffaloes that received RY compared to those animals which received the control diet (8500 vs. 10750 numbers/ml).

Feeding RY to lactating buffaloes did not affect ($P>0.05$) total tract DM and CP digestibility (Table 2).

However, NDF and ADF digestibility was significantly (P<0.05) higher in buffaloes fed the diet with RY compared to the control group.

Results regarding rumen fluid parameters are shown in Table 3. The level of rumen ammonia-N was lower (P<0.05) while total VFAs concentration was higher

Table 1: Effect of rumen yeast (*Saccharomyces cerevisiae*) on dry matter intake, milk production and milk composition in Nili-Ravi lactating buffaloes

Parameters	Without RY (control, n=8)	With RY (treated, n=8)	P-value
Average body weight (kg/head)			
Initial weight (kg)	541.50 ± 12.33	514.60 ± 15.49	0.252
Final weight (kg)	558.25 ± 10.09	545.50 ± 11.45	0.361
Total weight gain (kg)	16.75 ± 8.09	30.90 ± 10.75	0.039
Total DM intake (kg/head per day)			
Concentrate	2.70 ± 0.00	2.70 ± 0.00	0.471
Maize silage*	11.60 ± 0.50	12.00 ± 0.46	0.353
Total	14.30 ± 0.50	14.70 ± 0.46	0.372
Milk production (L/day)			
Milk yield	9.15 ± 0.26^{b}	9.60 ± 0.22^{a}	0.041
4% Fat corrected milk	11.32 ± 5.00^{b}	11.85 ± 5.00^{a}	0.037
Milk composition (%)			
Protein	4.42 ± 0.24	4.58 ± 0.18	0.293
Fat	6.35 ± 0.19	6.55 ± 0.15	0.257
Solids non fat	9.99 ± 0.29	9.91 ± 0.22	0.187
Total solids	16.34 ± 0.27	16.46 ± 0.30	0.328
Initial somatic cell count (no per ml)	13165 ± 87.5	12950 ± 98.7	0.401
Final somatic cell count (no per ml)	10750 ± 113.2^{b}	8500 ± 137.4^{a}	0.007

Mean±SE with different superscripts in the same row differ significantly (P<0.05). * Maize silage was offered *ad libitum* as a sole forage source plus 3 kg concentrate (16% CP and 72% TDN) daily without RY serves as control and with RY (14 g/head per day) as treated. n=8 Nili-Ravi lactating buffaloes per treatment

Table 2: Effect of rumen yeast (*Saccharomyces cerevisiae*) on nutrients intake and digestibility in Nili-Ravi lactating buffaloes

Description	Without RY (control, n=8)	With RY (treated, n=8)	P-values
Average daily intake (kg/day)			
Dry matter	14.60 ± 0.50	14.70 ± 0.46	0.117
Crude protein	1.38 ± 0.06	1.40 ± 0.04	0.251
Neutral detergent fibre	2.83 ± 0.11	2.89 ± 0.10	0.368
Acid detergent fibre	2.13 ± 0.11	2.16 ± 0.10	0.303
Nutrients digestibility (%)			
Dry matter	70.98 ± 1.31	69.88 ± 0.64	0.265
Crude protein	68.25 ± 1.17	67.99 ± 0.34	0.419
Neutral detergent fibre	59.91 ± 0.78^{b}	62.72 ± 0.97^{a}	0.041
Acid detergent fibre	54.23 ± 0.56^{b}	56.87 ± 0.67^{a}	0.036

Mean±SE with different superscripts in the same row differ significantly (P<0.05)

Table 3: Effect of rumen yeast (*Saccharomyces cerevisiae*) on rumen fermentation parameters in Nili-Ravi lactating buffaloes

Description	Without RY (control, n=8)	With RY (treated, n=8)	P-values
pH	7.32 ± 0.50	7.04 ± 0.46	0.141
Ammonia nitrogen (mg/ml)	14.87 ± 0.06	11.63 ± 0.04	0.037
Total VFAs (mg/ml)	2.13 ± 0.11	2.65 ± 0.10	0.031

Mean±SE with different superscripts in the same row differ significantly (P<0.05)

Table 4: Economic analysis of rumen yeast (*Saccharomyces cerevisiae*) supplementation to Nili-Ravi lactating buffaloes

Description	Without RY (control, n=8)	With RY (treated, n=8)
Cost of feed ($/day)		
Concentrate	0.87	0.87
Maize silage	2.74	2.77
Rumen yeast	-	0.05
Total feed cost ($/head per day)	3.61	3.69
Average milk yield (L/head per day)	9.15	9.60
Market price of milk produced ($/head per day)	6.59	6.91
Economic benefits (output/input)	1.83	1.88

(P<0.05) in buffaloes receiving RY supplemented diet compared to the control group.

The economic benefit from feeding RY supplement to lactating buffaloes is shown in Table 4. On DM basis, the price of one kg maize silage was $ 0.23, whereas the cost of concentrate was $ 0.32. The market price of each litre of milk was considered as $ 0.72. Therefore, total feed cost with RY was $ 3.69 per buffalo per day and control feed cost was $ 3.61. Market price of daily milk produced per buffalo with RY was $ 6.91, and for the control buffalo was $ 6.59. Therefore, economic benefit (ratio of output/input) with RY had a slightly higher effect compared to control (1.88 vs. 1.83).

Discussion

The positive effect of RY supplementation on post-partum (35 to 50 days) BW changes of Nili-Ravi lactating buffaloes compared to the control group might be due to the greater availability of energy that might had resulted in body restoration. The RY supplementation might have stimulated the growth of cellulolytic bacteria which resulted in higher NDF digestibility and more production of VFAs for energy (Ayad *et al*., 2013) which were also recorded in this study. Degirmencioglu *et al*. (2013) also observed a decrease in mobilization of body reserves in yeast supplemented buffaloes than control group, however the effect was not significant.

No effect of RY on DMI of buffaloes, in present study, was in accordance with the findings of previous researchers who reported that live yeast supplementation has no effect on DMI in dairy cattle (Rossow *et al*., 2017) and heifers (Ghazanfar *et al*., 2015). But, others found the higher (P<0.05) DMI in dairy cattle (Desnoyers *et al*., 2009), Anatolian buffalo (Degirmencioglu *et al*., 2013) and sheep (Payandeh and Kafilzadeh, 2007) receiving yeast supplementation. The inconsistency in the results may be due to the difference in feed type, feed intake, age, health and stress status of animals which may affect yeast efficacy (Moallem *et al*., 2009).

The positive effect of RY on milk production in present study was in line with the findings of previous researchers (Stein *et al*., 2006; Moallem *et al*., 2009; Degirmencioglu *et al*., 2013) for cows, and may be attributed to an increase in NDF digestibility and more VFA production thus allowing higher energy availability for milk yield. The lower SCC in the buffaloes fed with supplemented-RY diet compared to the control group was in agreement with other studies (Stein *et al*., 2006; Sretenović *et al*., 2008). The researchers noted that the reduction of SCC in yeast supplemented cows may be attributed to a better health status of udder (Stein *et al*., 2008) or the improved immune status of these cows (Bakr *et al*., 2015). But the probable mechanisms involved are not yet clear.

The increase in NDF and ADF digestibility with RY supplementation in present study might be due to enhanced cellulolytic bacteria population, which resulted in higher utilization of cellulose and more production of VFAs for energy. Moallem *et al*. (2009) also observed improvement in fibre digestion with addition of yeast to the diet because of the increase in the number of cellulolytic bacteria. Mosoni *et al*. (2007) reported higher cellulolytic bacteria population in the rumen of sheep fed a diet supplemented with live yeast culture. Guedes *et al*. (2008) concluded that live yeast supplementation has improved fibre digestibility up to 4.3% in cows fed corn silage based diets that supports our NDF and ADF digestibility results in buffaloes. However, no increase in DMD was observed with RY in buffaloes. Increase in NDF digestibility without any increase in DMD in RY fed cows compared to control was also observed by Bitencourt *et al*. (2011) and Pinloche *et al*. (2013) however, the reason was not clearly known.

The higher total ruminal VFAs concentration in RY fed group than the control animals, in our study, was in line with the findings of Abd el-Tawab (2007) and Bakr *et al*. (2015). The increase of total VFAs concentration in RY fed buffaloes might be due to more NDF digestibility. More VFA (especially propionate) production results in reduced availability of H_2 and C, required for methane production, and consequently reduces energy loss (Bakr *et al*., 2015). In our study, rumen ammonia-N concentration was significantly lower in RY treated buffaloes than the control animals which is in agreement with previous findings (Abd el-Tawab, 2007; Moallem *et al*., 2009; Bakr *et al*., 2015). The decrease in rumen ammonia-N of RY fed buffaloes appear to be the result of incorporation of ammonia into microbial protein (Bakr *et al*., 2015), or it may be due to inhibitory effect of RY on proteolysis (Khattab *et al*., 2003).

In conclusion, dietary supplementation of lactating buffaloes with RY had positive effects on milk production, milk SCC, NDF digestibility and VFAs concentration in Nili-Ravi buffaloes fed on maize silage-concentrate based diet.

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Conflict of interest

There is no potential conflict of interest by the authors for this research paper.

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