Effect of *Saccharomyces cerevisiae* Live Cells on *In Vivo* Digestibility and Nitrogen Excretion in Lactating Buffaloes

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Abstract: The effects of dietary inclusion of *Saccharomyces cerevisiae* culture on intake, *in vivo* digestibility, and fecal nitrogen excretion were examined in dairy buffalo. Forty lactating buffalo cows were equally divided into Control and Saccharomyces groups, balanced for milk production, parity, and days in milk. Two subsequent 16-d experimental phases were carried out. For both groups during the first experimental period a TMR based on maize silage (maize-TMR) was used, whereas in the second period an alfalfa haylage (alfalfa-TMR) was administered to the animals. In each experimental period, Saccharomyces group was supplemented with 50 g/head/day of yeast (Biocell®, Limena, Padova, Italy), corresponding to 20×10^9 CFU/head/day *Saccharomyces cerevisiae* NCYC Sc47 strain. The yeast supplement was top-dressed onto the morning feed. Dry matter intake (DMI) was assessed for 6 consecutive d on group basis, by the difference between feed offered and refused. In the last 3 days of experimental period *in vivo* digestibility was determined by using acid-insoluble ash (AIA) as an intrinsic digestibility marker. Saccharomyces supplemented buffalo cows presented greater DMI of maize-TMR, whereas no statistical differences between the groups were observed for alfalfa-TMR. Saccharomyces supplementation significantly improved *in vivo* digestibility of both TMR. Fecal nitrogen excretion was significantly reduced by the use of yeast supplementation. Results suggest that the inclusion of *Saccharomyces cerevisiae* culture in the diet for lactating buffaloes can be recommended for its effects on cow's digestive efficiency and fecal nitrogen excretion.

Keywords: Buffalo cows, Yeast, Dry matter intake, Digestibility, Fecal nitrogen excretion.

INTRODUCTION

In Italy dairy water buffalo (*Bubalus bubalis* L.) farming is concentrate in fertile coastal plains of Campania and Lazio Regions where, together with horticulture, it has a significant role in agriculture economy. The economic importance of buffalo sector is largely due to consumer demand for *Mozzarella di Bufala Campana* (MBC), a high quality, unripened cheese, that since 1996 received the European Certification PDO (Product of Designated Origin, EEC Regulation n. 1107). The annual sales turnover of MBC has been steadily growing and at 2011 stands at around 500 million Euros [1]. Statistical data from Agricultural Census 2010 [1] indicate in Italy 358,341 buffalo heads. Despite this relatively low number of animals compared to other countries, milk production of Italian Mediterranean buffaloes is one of the highest in the world, with an average of 2,218 kg/270 d lactation [2].

Notwithstanding these strengths, nowadays buffalo farmers are facing many constraints, including reducing milk prices, rising feeding costs, and the effects of nitrate pollution risks of groundwater that may limit their feed production [3]. As matter of the fact, many of the most important areas of buffalo farming are currently classified as *Nitrate vulnerable zones* by Italian legislation, in compliance with the European Union Nitrate Directive 91/676/EEC concerning the protection of waters against pollution caused by nitrates from agricultural source.

In such a context the improvement of digestive efficiency in lactating buffaloes is a matter of great interest.

The valorization of feed sources by ruminants is influenced by biochemical and microbial characteristics of the rumen environment. Thus, rumen ecosystem plays a key role in ruminants' response to their diet[4]. A number of studies [4-6] indicate that dietary supplementation with specific strains of *Saccharomyces cerevisiae* may influence rumen fermentations and thus may have an impact on the productive performance and diet digestibility.

Aim of this research was to examine the effects of dietary inclusion of *Saccharomyces cerevisiae* culture on intake, *in vivo* digestibility and fecal nitrogen excretion of lactating buffaloes.

MATERIALS AND METHODS

Experimental Design, Animals and Diets

The experiment took place from May to July 2013 at a commercial buffalo farm located in the Sele plain, Campania Region, southern Italy.

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Forty lactating buffalo cows were equally divided into Control (C) and Saccharomyces (S) groups, balanced for parity (Mean ± SD. 3.15 ± 2.01 vs. 3.3 ± 1.89 number of calvings, respectively), days in milk (DIM) (93.6 ± 43.8 vs. 91.2 ± 41.0 d) and milk production (10.5 ± 1.35 vs. 10.3 ± 1.56 kg/head/d). Groups were housed in two adjacent free stall pens with concrete floor and equipped with concrete feed manger, drinking bowls and partially sheltered outdoor paddock. Animals were managed in similar way in terms of feeding and management. They were milked twice daily at 0530 and 1700 h.

In order to assess whether the two groups had similar dry matter intake (DMI) and *in vivo* digestibility, the experiment was preceded by a 16-d pre-experimental period where both groups received a total mixed rations (TMR) based on maize silage (maize-TMR) without any yeast supplementation.

Subsequently, two 16-d experimental periods followed directly the pre-experimental phase. For both groups during the first experimental period a TMR based on maize silage (maize-TMR, the same as the pre-experimental period) was used, whereas in the second period a TMR based on alfalfa haylage (alfalfa-TMR) was administered to the animals. The two TMR were the same as those used by the farmer for buffalo cows producing 10 kg milk/d. In each experimental period, S group was supplemented with 50 g/head/day of yeast (Biocell®, Limena, Padova, Italy), corresponding to 20×10^9 CFU/head/day *Saccharomyces cerevisiae* NCYC Sc47. The yeast supplement was top-dressed onto the morning feed.

Cows were fed once daily (0800 h) in a given amounts to provide approximately 10% orts for ad libitum consumption. The TMR was pushed up several times daily to ensure unlimited access to feed.

Sampling and Measurements

In each period (one pre-experimental and two experimentals) after 10 d of adaptation to diet, DMI was assessed for 6 consecutive d on group basis, by the difference between feed offered and refused. Samples of feed and orts were collected daily and partial DM was determined after drying at 65°C until constant weight. The dried samples were composed across days and by group and analyzed for chemical composition.

In the last 3 days of each period (one pre-experimental and two experimentals) *in vivo* digestibility was determined by using acid-insoluble ash (AIA) as an intrinsic digestibility marker [7]. Fecal grab samples from each animal were collected at 0900, 1200 and 1700 h. A daily subsample was used to determine nitrogen content and the remaining was dried at 65°C to determine partial DM. The dried samples were composited across days and by cow, for a total of 40 samples/diet, and analyzed for chemical composition.

The AOAC official methods [8] were used to determine ash, ether extract and CP contents of composited samples of feed, orts and feces. The organic matter (OM) content was calculated as the difference between DM and ash contents. Neutral detergent fiber (NDF), acid detergent fiber (ADF) inclusive of residual ash, and acid detergent lignin (ADL) were determined by methods of Van Soest *et al*. [9]. Sodium sulfite was added during NDF extraction and a heat-stable α-amylase was used when necessary. Starch was determined by using a Polax-2l polarimeter in 200 mm long observation tubes. AIA was assessed by the 2 N hydrochloric acid procedure of Van Keulen and Young [10].

Net energy for milk production was calculated from the chemical composition of feedstuffs [11]. Fecal nitrogen excretion was estimated on the basis of DM intake, DM digestibility, and the nitrogen fecal content.

Statistical Analysis

Data were analyzed with GLM procedure of the Statistical Analysis System package [12].

For the pre-experimental period, DMI, apparent digestibility coefficients (ADC), and fecal nitrogen excretion data were analyzed using a one-way analysis of variance model with group (Control vs. Saccharomyces) as factor.

DMI, ADC and fecal nitrogen excretion data collected during the two experimental periods were analyzed by a two-way analysis of variance with group (Control vs. Saccharomyces), diets (maize-TMR vs. alfalfa-TMR), and interaction as factors. The experimental unit was the day of sampling (i.e 1^{st} to 6^{th}) for DMI and the cow for ADC and fecal nitrogen excretion.

Effects of the factors were declared significant at $P<0.05$.

RESULTS AND DISCUSSION

The components and chemical composition of the two TMR are presented in Table **1**. Compared to alfalfa, maize -TMR had lower CP (17.2 vs. 19.1 % DM, for maize and alfalfa TMR, respectively), higher ENL (6.4 vs. 5.8 MJ/kg DM), starch (15.6 vs. 7.3 % DM) and NDF contents (NDF 50.3 vs. 47.5 % DM).

As expected, the chemical characteristics of diets with or without yeast supplementation were very close (data not shown).

Maize-TMR met the estimated nutritional requirements of buffalo cows yielding on average 10 kg/d of milk [13], with the exception of NDF (+8.3 percentage points, pp) and starch (- 4.4 pp) contents. By contrasts, alfalfa-TMR was deficient in energy concentration (-0.46 MJ/kg DM) and starch level (-12.7 pp), whereas crude protein exceeded the recommendation (+3.8 pp). Such imbalances were mainly due to the simultaneous use of alfalfa and protein feeds.

Table **2** shows the DMI, ADC and fecal nitrogen excretion of the Control and Saccharomyces groups measured during the pre-experimental period, when yeast supplementation was not administered. No significant differences were noted between the two groups.

The DMI, ADC and N excretion determined in the two experimental periods are reported in Table **3**. Except for DMI, the interaction diet x group was never significant.

DMI of maize-TMR was greater in S cows than in C cows (16.6 vs. 16.1 kg/head/d, respectively; SEM 0.06; $P<0.05$), whereas alfalfa-TMR intake did not significantly differ between groups (18.6 vs. 18.5 kg/head/d for groups S and C, respectively). The different response to yeast supplementation explains the significance of the interaction diet x group ($P< 0.0001$; Table **3**).

Table 1: Composition of the Two Experimental Total Mixed Rations (TMR)

	Maize TMR	Alfalfa TMR
Components, kg/head/d		
Maize silage	23.0	-
Alfalfa haylage	-	12.0
Mixed grass hay	3.5	2.5
Alfalfa hay	1.5	-
Alfalfa fresh	-	8.0
Maize distillers	4.0	3.2
Wheat Flour Middlings	1.0	4.5
Sunflower meal	2	1.0
Wheat straw	-	1.5
Vitamins and Minerals	0.12	0.13
Chemical Characteristics		
Dry matter, kg	16.7	19.0
ENL, MJ/kg DM	6.40	5.82
Crude protein, % DM	17.2	19.1
NDF, % DM	50.3	47.5
NDF roughage, % DM	35.5	33.1
ADF, % DM	28.6	29.6
ADL, % DM	4.6	4.3
Ether extract, % DM	5.2	4.5
Ash, % DM	6.6	8.2
Starch, % DM	15.6	7.3

Table 2: Least Square Means of Dry Matter Intake and Apparent Digestibility Coefficients (ADC) of Control and Saccharomyces Groups Determined During the Pre-Experimental Period

	Pre-experimental Maize TMR[1]		SEM	P value
	Control[1]	Saccharomyces[1]		
Intake, kg/head/d				
Dry matter	16.2	16.3	0.1	0.41
Crude protein	2.8	2.8	0.01	0.42
ADC, %				
Dry matter	60.6	60.9	0.8	0.82
Organic Matter	64.1	64.4	0.7	0.75
Crude Protein	58.5	58.8	0.9	0.80
NDF	51.0	51.4	1.0	0.76
ADF	34.1	35.3	1.3	0.54
Fecal N excretion				
g N /head/d	183.4	183.2	3.8	0.96

[1]In the pre-experimental period *Saccharomyces cerevisiae* supplementation was not administered.

Table 3: Least Square Means of Dry Matter Intake and Apparent Digestibility Coefficients (ADC) as Influenced by Yeast Supplementation and Diet

	Group		Diet		SEM	P value		
	Control	Saccharomyces	Maize-TMR	Alfalfa-TMR		Group	Diet	Diet x Group
Intake, kg//head/d								
Dry matter	17.3	17.6	16.4	18.5	0.04	0.0001	<.0001	0.0001
Crude protein	3.2	3.2	2.8	3.5	0.01	0.0947	<.0001	0.39
ADC, %								
Dry matter	59.1	61.1	61.9	58.2	0.4	0.003	<.0001	0.66
Organic Matter	62.6	64.4	65.3	61.6	0.4	0.0012	<.0001	0.5433
Crude Protein	58.9	61.7	60.4	60.2	0.7	<.0001	0.69	0.79
NDF	47.7	50.7	52.6	45.8	0.5	<.0001	<.0001	0.98
ADF	32.8	37.5	37.5	32.9	0.6	<.0001	<.0001	0.15
Fecal N excretion								
g N head/d	206.9	196.4	177.6	225.8	21.9	0.0011	<.0001	0.13

In literature contradictory results are reported. In studies on dairy cows a number of authors found a significant increase in DMI as a result of supplementation with yeast [14-18], while others [19-21] found no effects. Also in experiments on lactating buffaloes results in the literature are not univocal [22-24].

These conflicting reports may be explained by differences in supplementation (i.e. yeast strain and dosage), stage of lactations [15] and nutritional characteristics of basal diet. In this study during the two experimental periods the yeast supplementation and the DIM of cows were similar, whereas the nutritional characteristics of basal diets varied. It is well known that DMI is strongly influenced by dietary NDF [25]. In accordance with Wallace [26], it may be assumed that an improvement of rumen biomass activity by *Saccharomyces* resulted in an increased rate of cellulosolysis, a more rapid emptying of the rumen which determined a greater DMI of maize-TMR compared to Control group. A similar effect was not

observed with alfalfa-TMR probably due to the lower NDF content masking yeast effect on DMI (Table **3**). The significantly lower DMI of maize-TMR compared to alfalfa-TMR ($P < 0.001$; Table **3**) may be an indirect confirmation of this assumption.

Digestibility of DM, CP, NDF and ADF were significantly higher in group Saccharomyces than in group Control (Table **3**). These results agree with previous studies on buffalo cows [22, 23, 24] and calves [27].

In dairy cows, effects of yeast supplementation on digestibility are variable, and often unpredictable, and much remains to be established about the influence of diet. Greater apparent digestibility of DM or other nutrients in response to yeast supplementation were observed in several works [14, 15, 28-31], but not in others [18, 32, 33].

The action of *Saccharomyces cerevisiae* on ruminal fiber digestibility may be due to the presence of soluble growth factors (*e.g.*, organic acids, B vitamins, amino acids) and metabolic intermediates that selectively stimulate ruminal bacteria growth which utilize lactate and digest cellulose [34]. The yeast may positively influence also the action of fungi and protozoa that break down the chemical bonds between cellulose and lignin [5, 35]. Moreover, it has been suggested that the live yeast's capacity to scavenge oxygen contributes to create anaerobic conditions in the rumen, which is favorable to most of the ruminal microorganisms [5, 35].

Additionally, a number of authors [18, 36-38] have observed lower rumen ammonia concentrations as an effect of yeast supplementation, whereas others [38, 39] have not detected any effect. This reduction may indicate greater utilization of protein for the microflora synthesis that might contribute to increasing digestibility of nutrients.

Statistically significant differences were also found between diets, with the higher values observed for maize-TMR (Table **3**). These results are consistent with the composition of the two TMR. Alfalfa, like all legumes, has a high lignin to cellulose ratio, which weakens the cell wall structure and facilitates the formation of smaller and more hydratable particles. These conditions favor the microbial degradation, but also increase the rumen transit rate, causing, therefore, a reduction of digestibility [40, 41].

As an effect of the better CP digestibility, nitrogen excretion was lower in Saccharomyces than in Control group ($P<.0001$; Table **3**). On yearly basis, dietary yeast supplementation would reduce N fecal excretion of almost 4 kg N/year/lactating cow. The observed values of daily N fecal excretion are comparable, even if slightly higher, to those reported by others for lactating buffaloes [42, 43]. In these studies CP intake of cows was lower than that observed in the present work and this may explain the small discrepancy.

It must be underlined that the excess of dietary protein content of alfalfa-TMR led to a significant increment of nitrogen excretion as compared with maize-TMR ($P<0.0001$; Table **3**).

Nitrogen loss from manure represents one of the major environmental impacts of dairy production [44]. In Italy, the traditional areas of buffalo farming are mostly *Nitrogen Vulnerable Zones*, in which it is mandatory to reduce N surplus and loss at both farm and herd level. Within the herd, lower concentration of dietary N and the dietary supplementation with yeast may be a practical approach that can be easily applied by buffalo farmers.

CONCLUSIONS

In this study, live yeast supplementation to lactating buffaloes improved apparent total tract digestibility of two TMR (maize and alfalfa) which decreased the release of nitrogen in the environment . In addition, yeast supplementation increased the dry matter intake of the diet based on maize-TMR.

Results suggest that the inclusion of *Saccharomyces cerevisae* culture in the diet for lactating dairy buffalo cows can be recommended under field conditions.

ABBREVIATION

ADC = Apparent digestibility coefficients

ADF = Acid detergent fibre

ADL = Acid detergent lignin

AIA = Acid Insoluble Ash

CP = Crude Protein

DIM = Days in milk

DM = Dry matter

DMI = Dry matter intake

EEC = European Economic Community

GLM = General Linear Model

MBC = Mozzarella di Bufala Campana

N = Nitrogen

NDF = Neutral Detergent Fibre

OM = Organic Matter

PDO = Product of Designated Origin

TMR = Total mixed rations

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