Australian Journal of Basic and Applied Sciences, 1(4): 379-385, 2007
ISSN 1991-8178

Effect of *Saccharomyces cerevisiae* of Yeast on Fiber Digestion in Sheep Fed Berseem (*Trifolium alexandrinum*) Hay and Cellulase Activity

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Abstract: The current study was conducted to evaluate the effect of baker's yeast (*Saccharomyces* cerevisiae, SC) on the digestibilities of dry matter (DM), organic matter (OM), neutral detergent fiber (NDF), acid detergent fiber (ADF) and the degradation kinetics of NDF and ADF of berseem (*Trifolium* alexandrinum) hay. Three ruminally cannulated sheep were used in a 3x3 Latin square design experiment on three diets; (1) berseem hay (basal diet), (2) basal diet plus 11.25 g/head/d SC, and (3) basal diet plus 22.5 g/head/d SC. The effect of cell-free culture (CFC) of *Saccharomyces cerevisiae* on cellulase activity was investigated, in vitro, by using enzyme kinetic assay. Digestibilities of DM, OM, NDF and ADF of berseem hay were increased ($P<0.05$) when the supplementation level of SC was 22.5 g whereas 11.25 g had no effect. Degradation rate of NDF and ADF was enhanced ($P<0.05$) by the addition of SC at both levels of supplementation. Shorter lag time was noticed in the digestion of NDF and ADF at both levels of SC supplementation. The in vitro activity of cellulase was enhanced by the addition of CFC, whereas the maximum cellulase activity was observed with CFC from 6 days growth of the yeast culture. Interestingly, the cellulase activity was increased in a dose-dependent manner of CFC. In conclusion, baker's yeast enhanced cell wall degradation of berseem hay and dramatically reduced the lag time of digestion as a result of its direct enhancement of cellulase activity.

Keywords: Cell wall, digestion, degradability, yeast, *Saccharomyces* cerevisiae, berseem hay, sheep, cellulase activity

INTRODUCTION

During the past decades, ruminant nutritionists and microbiologists showed great interest in manipulating the microbial ecosystem of the rumen in order to improve the production efficiency based and as a solution for the growing concern over the use of antibiotics and other growth promoters in the animal feed industry. As a result, interest in the effects of microbial feed additives on animal performance has increased during the past 20 years. Dietary supplementation with dry yeast cultures (*Saccharomyces cerevisiae*) was noted to improve the digestibility of dry matter (DM), crud protein and hemicellulose by increasing the rumen bacterial number and outflow rate of microbial nitrogen post ruminally (Wiedmeier *et al.,* 1987; Wallace and Newbold 1992; El-Waziry *et al.,* 2000; El-Talty *et al.,* 2001; Marghany *et al.,* 2005). However, Adams *et al.,* (1981) observed little effect of yeast supplementation on ruminal pH, ruminal NH_3-N, VFA (volatile fatty acids) concentrations and fiber digestion. Similar results were reported by Newbold *et al.,* (1995) who found that the degradation of hay in the rumen of sheep fed a mixed forage; concentrate diets was not influenced by yeast supplementation over 72 hrs of the incubation period. Further, Kholif and Khorshed (2006) found no modification in NH_3-N concentration by the addition of yeast. More than 1000 strains of *Saccharomyces cerevisiae* are listed in American Type Culture Collection Catalogue (ATCC, 1990) and the apparent probiotic activity of these strains have not widely been investigated (Newbold *et al.,* 1995). Moreover, they found no differences on the concentrations of acetate, propionate, butyrate and NH_3-N, ruminal pH and the digestion of dry matter after 24 and 48 hrs when either hybrid baker's yeast or Yea-Sacc was included to the Rusitec. However, in a contradictory report the addition of yeast has been shown to decrease the concentration of NH_3-N in the rumen, as a result of its direct effect on reducing the degree of protein degradation (El-Waziry *et al.,* 2000; Kamel *et al.,* 2000; Eweedah *et al.,* 2005). However, there are little

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studies on the effect of yeast supplementation on the degradation of roughage when fed as the sole diet and no study was undertaken so far to elucidate the effect of yeast on cellulase activity and fiber digestion. In this study, we examined the effect of baker's yeast of *Saccharomyces cerevisiae* (SC) on the digestibilities of dry matter (DM), organic matter (OM), neutral detergent fiber (NDF) and acid detergent fiber (ADF), and ruminal degradation kinetics of DM, NDF and ADF of sheep fed berseem (*Trifolium alexandrinum*) as the hay sole diet. The effect of cell-free culture (CFC) of *Saccharomyces cerevisiae* on cellulase activity was also investigated, *in vitro,* by using enzyme kinetic assay.

MATERIALS AND METHODS

Animals and Diets:

Animal experiments were carried out at the experimental station of Faculty of Agriculture, Alexandria University, Egypt, and biochemical experiments were at the Department of Biochemistry & Biotechnology, Faculty of Agriculture, Kagoshima University, Japan. Three male sheep (live weight of 50 kg , S.E ± 1.5 kg) fitted with ruminal cannula were used in an 3x3 Latin square design after experiment Animals were fed berseem (*Trifolium alexandrinum*) hay as sole diet at the level of 3% of their body weight (basal diet), dietary treatments were basal diet, basal diet plus baker's yeast (*Saccharomyces cerevisiae, SC*, with 28% DM)at 11.25 g/head/day and basal diet plus SC at 22.5 g/head/day. Yeast was added daily before the morning meal via the rumen cannula. Animals were fed twice daily at 0800and 1600hr, and had free access to fresh water.

Digestibility Trials:

Digestibility trials conducted using three animals for each treatment which were separated in individual pens. The last 15 days of each preliminary period rams were equipped with bags fitted to the animals with harness for total fecal collection. During the collection period (5 days) accurate records were kept for individual feed intake. Total fecal excretion was collected once daily and 10% representative samples were dried at 60ºC over night and kept in sealed bags until analysis.

Rumen Degradability Measurements:

Two polyester bags (Swissnylon Monofilament, Switzerlland), 7 cm x15 cm and pore size of 45 µm were used at each incubation time. Approximately 3 g of hay (ground to 2 mm) were placed in each bag. Bags were incubated in the rumen of each sheep and removed after 3, 6, 12, 24, 48 and 72 h, bags were rinsed and manipulated in cold water until the water ran clear, then squeezed prior to storage at - 20°C. Later, bags were thawed and washed again in running water as described by Kamel *et al.,* (1995) to eliminate the microorganism attached to residual hay. Two bags were washed in running water for 15 min to determine the initial soluble fraction (a).

The DM, NDF, and ADF disappearances were studied by fitting the individual values to the equation $P = a+b\ (1-exp^{-ct})$ proposed by Ørskov and McDonald (1979), and lag time (Lt) was determined as described by McDonald (1981), where P is the disappearance after time t, Lt is the lag time until the star of the degradation. The a, b and c are least squares estimated for soluble fraction, degradable fraction and the rate of degradation, respectively.

Nitrogen, DM and organic matter (OM) were determined according to A.O.A.C. (1990) NDF and ADF were determined according to Goering and Van Soest (1970).

Enzyme Kinetic Assay:

Preparation of Yeast (Saccharomyces cerevisiae)*:*

One mg dry of *Saccharomyces cerevisiae* was dissolved in 1 ml H^2O, incubated at 30ºC for 30 min, and diluted to 10^3 and 10^4 in H_2O and then 5 0 µl was Spread onto YEPDA agar plate (yeast extract 0.25 g, glucose 0.50 g, polypeptone 0.25 g and agar 1.00 g dissolved in 50 ml H_2O) and incubated at 28ºC for 3 days.

Preparation of Yeast Carbon Base (YCB) Medium for Yeast Growth:

L-Asparagine (0.20 g), 1 ml Trace elements, 10 ml Salts, 77 ml H_2O were autoclaved 121ºC for 20 min and cooled at room temperature. One ml 10% glucose (autoclaved), 10 ml 50% glycerol (autoclaved), 1 ml vitamins, 10 µl histidine, 20 µl methionine and 20 µl tryptophane were added to the cool solution.

Yeast Growth:

One Colony of yeast was suspended in 400 µl of sterile H_2O (solution 1) and then 10 ml YCB were added to 100 µl of solution 1 (solution 2) and incubated at 29ºC for one week. Two hundred µl of solution 2 were added to 1.8 ml 0.85 % Saline per day for one week and read at 600 nm by Spectrophotometer (SmartSpec3000, Bio-Rad, Japan).

Preparation Cell-Free Culture (CFC) of Yeast (Saccharomyces cerevisiae):

One ml of *Saccharomyces cerevisiae* culture (solution 2) was centrifuged at 5000 rpm for 5 min, 4ºC and supernatant (CFC) was transferred to a fresh tube. The effect of CFC of *Saccharomyces cerevisiae* on cellulase activity was investigated, *in vitro,* by using enzyme kinetic assay. Carboxymethyl Cellulose (CMC, Nacalai Tesque, Inc., Kyoto, Japan) Sodium Salt (Nacalai Tesque, Inc., Kyoto, Japan) was prepared as described by Becker *et al.,* (2001).

Procedure of Reaction:

The reaction procedure was done according to Becker *et al.,* (2001) as follows: 188.5 µl H_2O or medium or cell-free culture (CFC) or standard solution (0.04 mg glucose/ ml Na-acetate buffer dilute till limited amount of detection) was added to 65 µl Na-acetate buffer (pH 5.5), 6.5 µl Enzyme (cellulase, from *Asperigillus* niger, 1.18 U/mg Solid Nacalai Tesque, Inc., Kyoto, Japan), and 65 µl Substrate (CMC). The final volume of reaction mixture was 325 µl and then the mixture was incubated at 37ºC for 2h (Yuan *et al.,* 2001), followed by adding 163 ml glycine (1M, pH11.0, Nacalai Tesque, Inc., Kyoto, Japan): ethanol (94%, Nacalai Tesque, Inc., Kyoto, Japan) to stop the reaction (1 volume:9 volumes). The reaction mixture (200 µl) was mixed with 125 µl (0.1 M in 0.5 M NaOH) 4-hydroxybenzoic hydrazide (Aldrich Chemical Company, Inc., USA), boiled at 100ºC for 10 min and cooled in ice for 5 min., the solution was spin down and read absorption at 405 nm. Reducing sugars (glucose) were determined at the end of incubation period, and glucose solutions were used as standard equivalents of reducing sugars (Yuan *et al.,* 2001).

Statistical Analysis:

Results were analyzed by ANOVA for a 3x3 Latin square design using the JMP procedure of SAS (1994) and the model was $Y_{ijk} = \mu + A_i + T_j + P_k + e_{ijk}$, where: Y_{ijk} = observed response, μ = overall mean, $A_i = i^{th}$ animal, $T_j = j^{th}$ treatment, P_k = period, e_{ijk} =error term.

RESULTS AND DISCUSSIONS

There is no study was undertaken to elucidate the effect of yeast on cellulase and fiber digestibility so far. In this study, we examined the effect of yeast and cell-free culture (CFC) of *Saccharomyces cerevisiae* on fiber digestion and cellulase activity, respectively, to obtain the evidence of improvement of the fiber digestion by the addition of yeast.

Nutrients Digestibility:

The composition of hay on DM basis was 853.5, 24.0, 554.0 and 381.5 g/kg DM for OM, N, NDF and ADF, respectively. The added yeast diets had a slight increased in DM intake and digestibility coefficients for DM, OM, NDF and ADF are presented in Table 1. Digestibility of DM did not differ ($P>0.05$) significantly due to addition of *Saccharomyces cerevisiae (SC)* to the basal diet, however, it tended to be higher at the level of 22.5 g/d. The addition of SC increased ($P<0.05$) apparent digestibility of OM, NDF and ADF when the supplementation level was 22.5 g/d meanwhile, of 11.25 g/d had no effect. The general positive effect of SC addition apparent digestibility of OM in the present study is in agreement with results by Yoon and stern (1998) who observed an improvement in ruminal OM digestion and ruminal true OM digestibility in diet supplemented with yeast culture then that in unsupplemented diets. Also the present results of NDF and ADF digestibilities are supported by the findings of Wiedmeier *et al.,* (1987); Erasmus *et al.,* (1992); Harris *et al.,* (1992); Wholt *et al.,* (1991); Wholt *et al.,* (1998); Gado *et al.,* (1998); El-Talty *et al.,* (2001); Marghany *et al.,* (2005); Kholif *et al.,* (2005); Kholif and Khorshed (2006). They found a positive effect of yeast supplementation on digestion of ADF, NDF, cellulose, himecellulose and crude fiber, respectively. The benefits associated with *Saccharomyces cerevisiae* include increased DM and NDF digestion, may be attributed to the increase of numbers of rumen total viable bacteria and cellulolytic bacteria with animal fed on yeast (Newbold *et al.,* 1995; Lila *et al.,* 2004). Moreover, the stabilization of ruminal environment could be the reason for increase in total anaerobic bacteria and thus increase in cellulolytic bacteria when yeast cultures were added to mixed diets or ruminants (Wiedmeier *et al.,* 1987; Dawson and Newmen, 1988; Harrison *et al.,* 1988). Moreover, Williams *et al.,* (1991) suggested the action of yeast may be related partly to alleviation of negative associative effects of rapidly fermentable carbohydrate influencing cellulose digestion feeding hay as a sole diet generally maintains a high level of ruminal pH, which favors an increase in the ratio of acetate to propionate.

Table 1: Effect of bakery's yeast (*Saccharomyces cerevisiae, SC*) on digestibilities of dry matter (DM), organic matter (OM), neutral detergent fiber (NDF), acid detergent fiber (ADF) and DM intake in sheep fed berseem (*Trifolium alexandrinum*) hay as a sole diet (mean±SE)

Digestibility, %	Basal diet	Basal diet +11.25g/d SC	Basal diet +22.5g/d SC
DM	62.20±1.44	63.38±1.89	65.62±1.82
OM	63.71±0.75	63.93±1.15	67.45±0.63 [a]
NDF	60.20±0.55[b]	62.02±1.06 [b]	65.27±0.61 [a]
ADF	51.23±0.61 [b]	52.80±0.36 [b]	54.83±0.84 [a]
DM intake (g/day)	1406.0±54.6	1501.0±30.3	1498.0±44.8

Means in the same row with different letters in their superscript differ (P<0.05)

Table 2: Effect of bakery's yeast (*Saccharomyces cerevisiae, SC*) on the degradation Kinetics of dry matter (DM), neutral detergent fiber (NDF) and acid detergent fiber (ADF) in sheep fed berseem (*Trifolium alexandrinum*) hay as a sole diet (mean±SE)

Item	Basal diet	Basal diet + 11.25g/d SC	Basal diet +22.5g/d SC
DM			
a+b[1]	71.56±1.97[a]	75.67±0.79 [b]	75.58±1.06 [b]
u[2]	28.44±1.96[a]	24.33±0.74 [b]	24.42±1.09 [b]
c[1]	0.059±0.004 [b]	0.075±0.001[a]	0.076±+0.005 [a]
NDF			
b[1]	41.00±1.37	42.49±1.62	42.72±0.83
u[2]	59.00±1.32	57.51±1.61	57.28±0.82
c[1]	0.055±0.001 [b]	0.067±0.001 [a]	0.065±0.003 [a]
L[1]	6.83±0.29 [a]	4.64±0.59 [b]	3.67±0.55 [b]
ADF			
b[1]	39.10±0.56 [b]	40.10±0.67 [b]	42.43±0.30 [a]
u[2]	60.90±0.58 [a]	59.90±0.63 [a]	57.57±0.31 [b]
c[1]	0.044±0.001 [c]	0.053±0.003 [b]	0.060±0.001 [a]
L[1]	9.12±1.05 [a]	6.82±0.41 [b]	5.59±0.19 [b]

[1]Estimated by the equation of MacDonald (1981), where; a+b= degradable fractions (a= soluble fraction and b= slowly degradable fraction); c= degradation rate; L, lag time.
[2]Undegrable fractions {u= 100- (a+b)}.
[a,b,c] Means in the same row with different letters in their superscripts differ (P<0.05).

Table 3: Effect of cell-free culture (CFC) of *Saccharomyces cerevisiae* doses on cellulase activity[*]

	Without	With	Enhancement Rate (ΔΔ)
Doses (µl)	CFC		
0	0.330	0.330	0
20	2.148	2.170	0.022
40	2.636	3.898	1.262
80	1.818	3.591	1.773
100	2.114	3.455	1.341

[*]Expressed as µg/ml glucose which released in the medium per one hour.
Enhancement Rate (ΔΔ)=cellulase activity with CFC-without CFC

Degradation of Dry Matter and Cell Wall Constituents:

Ruminal degradation of DM, NDF and ADF are presented in Table 2. The degraded fraction (*a+b*) and the degradation rate (*c*) of DM was significantly enhanced (P<0.05) when yeast was added at both levels of supplementation, and no significant differences were found among the two levels of treatments. The values were 0.059 0.075 and 0.076/hr for basal diet, the 11.25 g/d and 22.5 g/d levels, respectively. No significant difference was found in the *b* fraction of NDF due to the addition of *SC*, but the b fraction of ADF was significantly increased (P<0.05) when SC was added at the level of 22.5 g/d. The values of c for basal diet, basal diet plus yeast at level of 11.25 and 22.5 g/d were 0.055, 0.067 and 0.065/hr for NDF and 0.044, 0.053 and 0.060/hr for ADF, respectively (Table 2). Reduction of lag time (*L*) was noticed in the digestion of NDF and ADF at both levels of yeast supplementation. The in situ results showed that the *SC* caused improve in the degradation of NDF and ADF, as a result of higher *c* and lower *L* (Table 2). The reduction of *L* in the degradation of NDF and ADF is a result of an increase in the mass of ruminal microorganisms, which would accelerate the degradation rate of NDF and ADF. This finding is in agreement with the results of Williams *et al.,* (1991). They found that yeast supplementation for dry cows growing steers decrease the lag time or the degradation of cell wall in mixed diet. The addition of *SC* in both levels was decreased undegradable fraction (*u*) of DM, NDF and ADF, as a result of an increase in the degradation rate and reduction of lag time, may be due to the increase of numbers of rumen total viable bacteria and cellulolytic bacteria with animal fed yeast (Newbold *et al.,* 1995; Lila *et al.,* 2004).

Enzyme Kinetic Assay:

First, we examined the presence of cellulase enzyme in yeast used in this study. The results showed negative cellulase activities in intracellular extract or secreted in the medium.

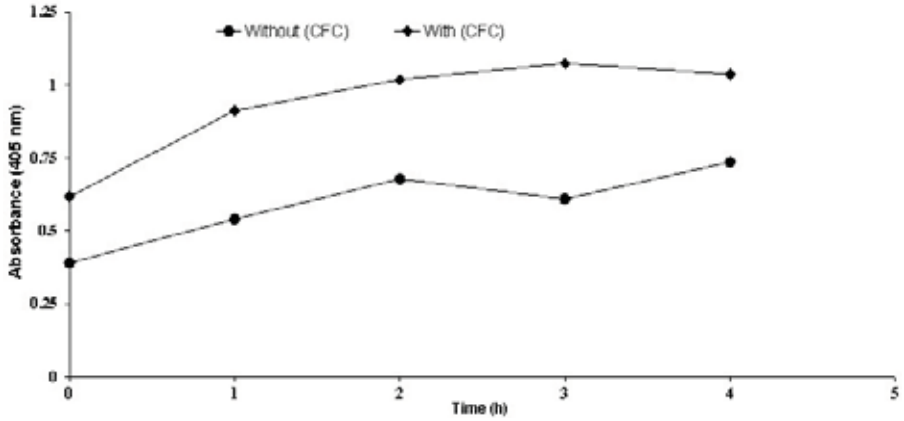

Fig. 1: Cellulase activity with or without cell-free culture (CFC) of *Saccharomyces* cerevisiae.

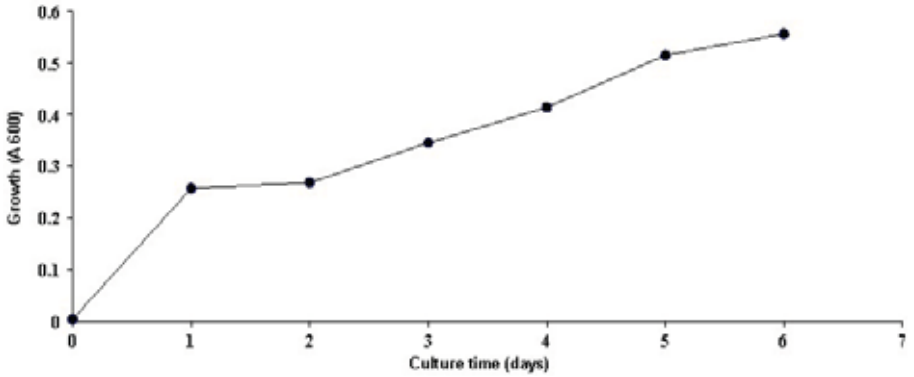

Fig. 2: Cellulase activity with or without cell-free culture (CFC) of *Saccharomyces* cerevisiae during 6 days growth of CFC.

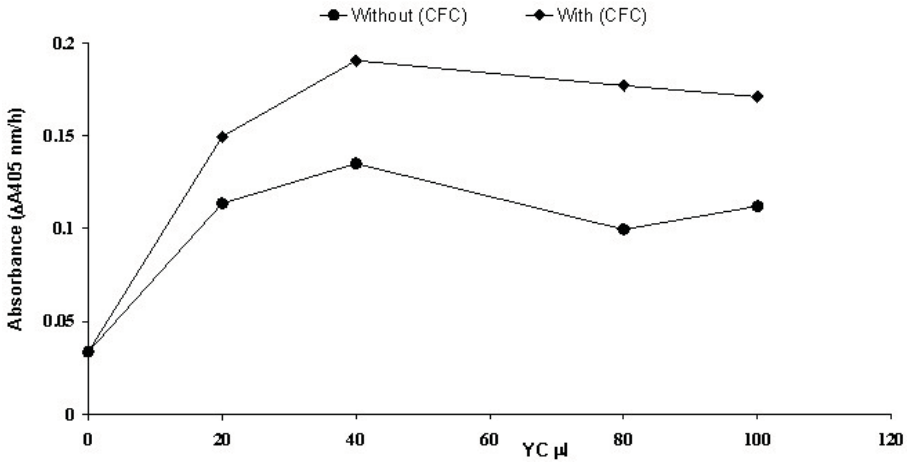

Fig. 3: Cellulase activity with or without different doses of cell-free culture (CFC) of *Saccharomyces cerevisiae*.

The in vitro activity of cellulase was enhanced by the addition of cell-free culture (CFC) during 4h incubation (Fig. 1). The yeast culture was incubated at 29ºC for one week, the maximum enhamcement of cellulase activity was observed with CFC from 6 days growth of the yeast culture (Fig. 2). Fig. 3 shows the incubation of cellulase with different doses of CFC (0, 20, 40, 80 or 100 µl of CFC). Interestingly, the cellulase activity was increased in a dose-dependent manner of CFC, and the maximum cellulase activity was detected with the addition of 80 µl CFC (Fig. 3 and Table 3). The enhancement rate of cellulase activity as glucose concentration was ranged from 0.022 to 1.773 µg/ml by the addition of 20 or 80 µl of CFC, respectively. Cellulolytic organisms secrete a number of individual cellulases, including exocellulases and endocellulases, which synergistically degrade crystalline cellulose to soluble oligosaccharides, primarily cellobiose. These oligosacchrides are then hydrolyzed to glucose by cellobiase (Wilson, 2003). The microbial degradation of cellulose and other fiber must be improved to be a more effective and profitable use of crop residues and other fibrous materials for the production of animals such as meat and milk. The present results showed that the addition of yeast to hay improved the microbial degradation as a result of an increase of degradation rate and reduction of lag time of cell wall of hay. The results provide an evidence, for the first time, that yeast addition to hay increases the cellulase activity in vitro and fiber digestion in vivo.

In conclusion, the results obtained form this study indicated that, the addition of 22.5 g/d baker's yeast to hay diet is recommended in the practical feeding on the sole berseem hay diet, as it considerably improved nutrition digestibilities, cell wall degradation and enhanced the outflow of ruminal microbes in the post-ruminal tract, and dramatically reduced the lag time of digestion as a result of its direct enhancement of cellulase activity.

AKNOWLEDGEMENTS

Dr. Ahmed M. El-Waziry is grateful to the Association of International Education, Japan (AIEJ) for financial support of the second part of this work at Kagoshima University, Japan.

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