

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

¹Ahmed M. El-Waziry and ²Hisham R. Ibrahim

¹Department of Animal Production, Faculty of Agriculture,
Alexandria University, Alexandria, Egypt.

²Department of Biochemistry & Biotechnology,
Faculty of Agriculture, Kagoshima University, Japan.

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), crude 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 $\text{NH}_3\text{-N}$, 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 $\text{NH}_3\text{-N}$ 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 $\text{NH}_3\text{-N}$, 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 $\text{NH}_3\text{-N}$ 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

Corresponding Author: Ahmed M. El-Waziry, Department of Animal Production, Faculty of Agriculture, Alexandria University, Alexandria, Egypt.
E-mail: aelwaziry@yahoo.com
"The Two Authors Contributed Equally to this Work."

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 \pm 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 0800 and 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 60°C over night and kept in sealed bags until analysis.

Rumen Degradability Measurements:

Two polyester bags (Swissnylon Monofilament, Switzerland), 7 cm x15 cm and pore size of 45 μ m 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 start 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 H₂O, incubated at 30°C for 30 min, and diluted to 10³ and 10⁴ in H₂O and then 50 μ l 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 H₂O) and incubated at 28°C 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 H₂O were autoclaved 121°C for 20 min and cooled at room temperature. One ml 10% glucose (autoclaved), 10 ml 50% glycerol (autoclaved), 1 ml vitamins, 10 μ l histidine, 20 μ l methionine and 20 μ l tryptophane were added to the cool solution.

Yeast Growth:

One Colony of yeast was suspended in 400 μ l of sterile H₂O (solution 1) and then 10 ml YCB were added to 100 μ l of solution 1 (solution 2) and incubated at 29°C for one week. Two hundred μ l 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, 4°C 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 H₂O 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 *Aspergillus 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 37°C 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 100°C 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, hemicellulose 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

¹Estimated 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.

²Undegradable 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 activity¹

Doses (µl)	Without	With	Enhancement Rate (ΔΔ)
	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

¹Expressed 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.

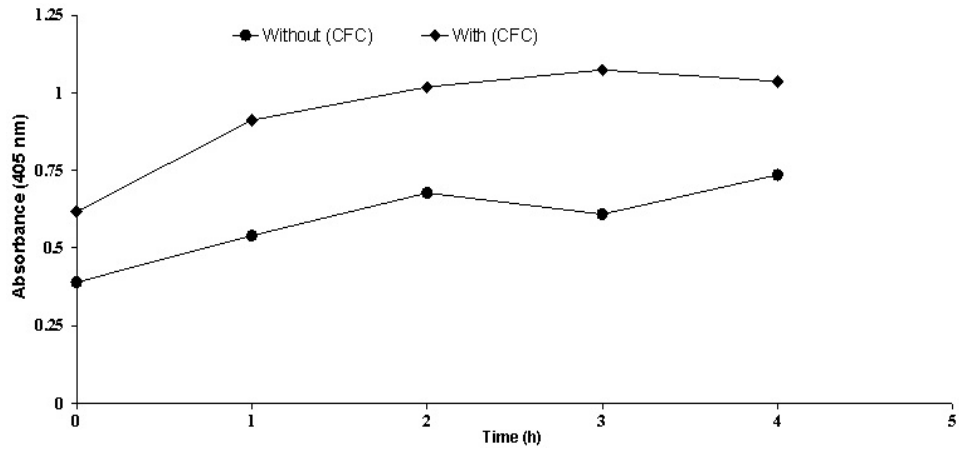


Fig. 1: Cellulase activity with or without cell-free culture (CFC) of *Saccharomyces cerevisiae*.

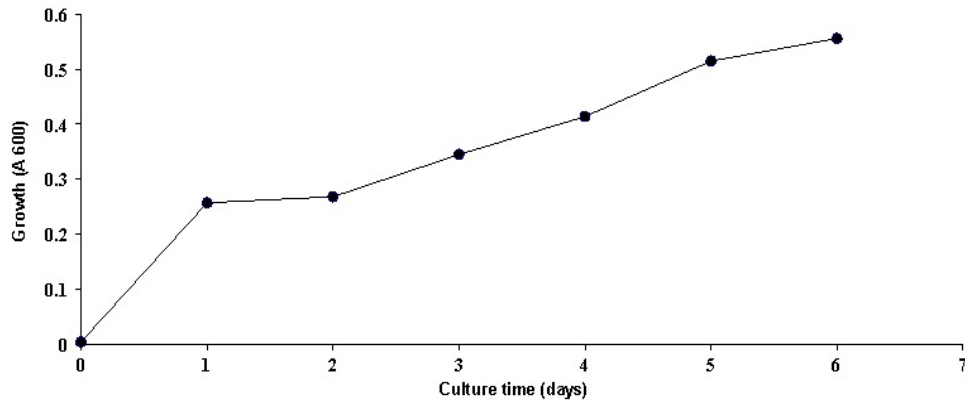


Fig. 2: Cellulase activity with or without cell-free culture (CFC) of *Saccharomyces cerevisiae* during 6 days growth of CFC.

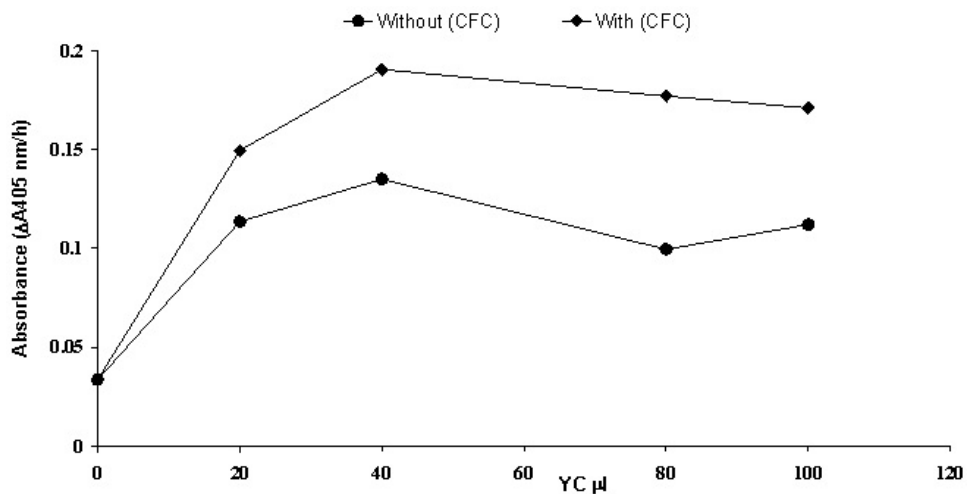


Fig. 3: Cellulase activity with or without different doses of cell-free culture (CFC) of *Saccharomyces cerevisiae*.

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 29°C for one week, the maximum enhancement 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 oligosaccharides 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 from 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.

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REFERENCES

- Adams, D.C., M.L. Galyean, H.E. Kiesling, J.D. Wallace and M.D. Finkner, 1981. Influence of viable yeast culture, sodium bicarbonate and monensin on liquid dilution rate, rumen fermentation and feedlot performance of growing steers and digestibility in lambs. *J. Anim. Sci.*, 53: 780.
- AOAC., 1990. Official Methods of Analyses., 1(5) ed. AOAC, Arlington VA.
- Becker, D., C. Braet, H. Brumer, M. Claeysens, C. Divne, B.R. Fagerstrom, M. Harris, T.A. Jones, G.J. Kleywegt, A. Koivula, S. Mahdi, K. Piens, M.L. Sinnott, J. Stahlberg, T.T. Teeri, M. Underwood and G.W. Fahrt, 2001. *Biochem. J.*, 356: 19.
- Dawson, K.A. and K.E. Newman, 1988. Fermentation in rumen-stimulating continuous cutlers receiving probiotic supplements. *J. Anim. Sci.*, 66 (Suppl.) 500 (Abstract).
- El Hassan, S.M., C.J Newbold, I.E. Edwards, J.H. Topps and R.J. Wallace, 1996. Effects of yeast culture rumen fermentation, microbial protein flow from the rumen and live- weight gain in bulls given high cereal diets. *Anim. Sci.*, 62: 43.
- El-Talty, Y.I., G.M. Abdul-Aziz and A.M. Ali, 2001. Calcium hydroxide treatment of some organic wastes. 2. Effect of performance of growing goats without or with yeast supplementation. *Egyptian J. Nutrition and Feeds.*, 4: 415.
- El-Waziry, A.M., H.E.M. Kamel and M.H.M. Yacout, 2000. Effect of baker's yeast supplementation to berseem (*Trifolium alexandrinum*) hay diet on protein digestion and rumen fermentation of sheep. *Egyptian J. Nutrition and Feeds.*, 3: 71.
- Erasmus, L.J, P.M. Botha and A. Kistner, 1992. Effect of yeast culture supplement on production rumen fermentation, and duodenal digesta flow in dry cows. *J Dairy Sci.*, 75: 36056.
- Eweedah, N.M., M.K. Mohsen, M.I. Bassiouni, M.F. Ali, M.M. Khalafalla, 2005. Performance of lambs fed on rations containing soybean meal treated with formaldehyde and probiotics. 1. Feeding value, rumen fermentation and degradability. *Egyptian J. Nutrition and Feeds. (Special Issue)*, 8: 361.
- Gado, H.M., A.Y. El-Badawi, F.L.S. Helal and Sohair A. Nasr, 1998. Effect of yeast culture supplementation level on the growth performance of growing goats. *Arab Univ. J. Agric. Sci., Ain Shams Univ., Cairo*, 6(1): 123.
- Goering, H.K. and P.J. Van Soest, 1970. Forage fiber analysis (Apparatus, reagents and some applications) *Agric. Handbook no. 379. Agric. Res.Serv. USDA, Washington, DC.*

Harrison, G.A., R.W. Hemken, K.A. Dawson, R.J. Harmon and K.B. Barker, 1988. Influence of addition of yeast culture supplement to diets of lactating cows on ruminal fermentation and microbial populations. *J. Dairy Sci.*, 71: 2967.

Kamel, H.E.M., A.M. El-Waziry and J. Sekine, 2000. Effect of *Saccharomyces cerevisiae* on fiber digestion and ruminal fermentation in sheep fed berseem hay (*Trifolium alexandrinum*) as a sole diet. *Asian-Aus. J. Anim. Sci. (Supplement)*, 13: 139.

Kamel, H.E.M., J. Sekine, T. Suga, and Z. Morita, 1995. The effect of frozen-rethawing technique on detaching firmly associated bacteria from in situ hay residues. *Can J. Anim. Sci.*, 75: 481.

Kholif, A.M., M.A. El-Ashry, H.A. El-Alamy, H.M. El-Sayed M. Fadel and S.M. Kholif, 2005. Biological treatments of banana wastes for feeding lactating goats. *Egyptian J. Nutrition and Feeds*, 8: 149.

Kholif, S.M. and M.M. Khorshed, 2006. Effect of yeast or selenized yeast supplementation to rations on the productive performance of lactating buffaloes. *Egyptian J. Nutrition and Feeds*, 9: 193.

Kumar, U., V.K. Sareen and S. Singh, 1994. Effects of *Saccharomyces cerevisiae* culture supplement on ruminal metabolism in buffalo calves given a high concentrate diet. *Anim. Prod.*, 59: 209.

Lila, Z.A., N. Mohammed, T. Yasui, Y. Kurokawa, S. Kanda and H. Itabashi, 2004. Effects of a twin of strain of *Saccharomyces cerevisiae* live cells on mixed ruminal microorganisms fermentation in vitro. *J. Anim. Sci.*, 82: 1847.

Marghany, M., M.A. Sarhan, A. Abd El-Hey and A.A.H. El-Tahan, 2005. Performance of lactating buffaloes fed rations supplemented with different levels of baker's yeast (*Saccharomyces cerevisiae*) Egyptian *J. Nutrition and Feeds. (Special Issue)*, 8: 21.

McDonald, I., 1981. A revised model for the estimation of protein degradability in the rumen *J. Agric. Sci., Camb*, 96: 251.

Newbold C., X.B. Wallace, F.M. Chen, Macintosh, 1995. Different strains of *Saccharomyces cerevisiae* differ in their effects on ruminal bacterial numbers in vitro and in sheep. *J. Anim. Sci.*, 1811.

Newbold, C.J., F.M. Macintosh and R.J. Wallace, 1998. Changes in the microbial population of a rumen-simulating fermenter in response to yeast culture. *Can. J. Anim. Sci.*, 78: 241.

Ørskov, E.R. and I. McDonald, 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. *J. Agric. Sci.*, 92: 499.

Plata, F., G.D. Mendoza, J.R. Barcena-Gama, S. Gonzala, 1998. Effects of yeast culture (*Saccharomyces cerevisiae*) on neutral detergent fiber digestion in steers fed oat straw based diets. *Anim. Feed Sci. Technol.*, 49: 203.

SAS, Inc., 1994. *User's Guide: Statistics. Version 2 edition.* SAS Institute, Inc, Cary, NC.

Wallace, R.J. and C.J. Newbold, 1992. Probiotics for ruminants. In: R. Fuller (Ed.) *The Scientific Basis of the Probiotic Concept.* pp317. Chapman Hall, London.

Wiedmeier, R.D., M.J. Arambel and J.L. Walters, 1987. Effect of yeast culture and *Aspergillus oryzae* fermentation extract on ruminal characteristics and nutrient digestibility. *J. Dairy Sci.*, 70: 2063.

Williams, P.E.V. and C.J. Newbold, 1990. Rumen probiosis: The effects of novel microorganisms on rumen fermentation and ruminant productivity. In: W. Hresign and D. J.A.Cole (Ed.) *Recent Advances in Animal Nutrition.* pp: 211-227. Butterworths, London.

Williams, P.E.V., A. Walker and J.C. MacRae, 1990. Rumen probiosis: The effects of addition of yeast culture (viable yeast {*Saccharomyces cerevisiae* + growth medium}) on duodenal protein flow in wether sheep. *Proc. Nutr. Soc.*, 49: 128A(Abst.).

Williams, P.E.V., C.A.G. Tait, G.M. Innes and C.J. Newbold, 1991. Effects of the inclusion of yeast culture (*Saccharomyces cerevisiae* plus growth medium) in the diet of dairy cows on milk yield and forage degradation and fermentation patterns in the rumen of steers. *J. Anim. Sci.*, 69: 3016.

Wilson, D.B., 2003. Microbial cellulose degradation mechanisms. *Proceeding of Mie Bioform, Biotechnology of Lignocellulose Degradation and biomass Utilization.* November, 10-14, Ise-Shima, Japan, 19.

Wohlt, J.E., T.T. Corcione and P.K. Zajak, 1998. Effects of yeast on feed intake and performance of cows fed diets based on corn silage during early lactation. *J. Dairy Sci.*, 81: 1345.

Yoon, I.K. and M.D. Stern, 1996. Effects of *Saccharomyces cerevisiae* and *Aspergillus oryzae* on ruminal fermentation in dairy cows. *J. Dairy Sci.*, 79: 411-417.

Yuan, S., Y. Wu and D.J. Cosgrove, 2001. A Fungal Endoglucanase with plant cell wall extension. *Plant Physiology*, 127: 324.