# EFFECTS OF LIVE YEAST (*Saccharomyces cerevisiae*) SUPPLEMENTATION ON NUTRIENT DIGESTIBILITY, RUMEN FERMENTATION AND RUMEN MICROBIAL POPULATION COUNT IN SHEEP

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**SUMMARY**

*This study investigated the impacts of dietary yeast culture (Saccharomyces cerevisiae) supplementation on rumen fermentation, nutrient digestibility and rumen microbial population in sheep. Three digestibility trials were carried out using fifteen Sohagi rams randomly assigned into three groups (5 rams/ each).Each trial lasted for three weeks, the firsttwo weekswere considered as a preliminary period followed by one week collection period.The groups were a control group fed basal diet without yeast supplement and treated groups fed either 0.5 (T1) or 1% (T2) yeast culture (YC) mixed with concentrate diet. All animals were fed 60% of their requirements as concentrate mixture while, chopped corn stover was given as roughages ad libitum. The results pointed out, the digestibility of dry matter, organic matter, crude protein, crude fibre, neutral detergent fibre and hemicellulose were significantly (P<0.05) improvedfor yeast treated diets compared with basal diet.Live yeast culture either 0.5or 1% in concentrate diet increased (P <0.05) the pH,volatile fatty acids (VFAs), acetate and propionate, while rumen ammonia nitrogen on centration was decreased (P<0.05). Live yeast supplementation (0.5 or 1%) improved (P<0.05) N retention.The rumenal bacterial and protozoal counts increased (P<0.05) due to YC supplementation. In conclusion, dietary live yeast culture (S. cerevisiae) particular1% of concentrate mixture may improve nutrient digestibility, fermentation patterns and rumen microbial population.*

***Keywords: Sheep, live yeast, nutrients digestibility, rumen fermentation parameters***

## INTRODUCTION

Fattening lambs demand diets with high concentrate ratio for increasing its growth performance (Mungoi *et al*., 2012). However, a highly concentrated diet tended to increase rumenal acidosis (Gonzalez-Momita *et al*., 2009). Supplementation of yeast to rumenant rations modified rumenal pH, consequently reduced the adverse effects of high-concentrate diet (Calsamiglia *et al*. (2012). In fact, high rumenal pH due to *Saccharomyces cerevisiae* supplemented to diets, increased numbers of cellulolytic bacteria, like *Fibrobacter succinogenes* and *Rumenococcus albus*, which raised rate of rumenal fiber digestion (Callaway and Martin, 1997),modified volatile fatty acids concentrations, moreover it increased pH and increasing quantity, of lactate utilizing bacteria, particularly *Selenomonas rumenantium* and *Meganosphera elsdenii* then decreased lactate accumulation in the rumen (Callaway and Martin, 1997).

Yeast supplementation enhances the release of energy in the rumen to be more accessible for growing microorganisms and provides nutrients and/or soluble growth factors for more rumenal flora (Brossard *et al*., 2006). The advantages of yeast culture supplement were changing rumenal fermentation, improved bacterial activity, and increased rumen ciliates, which enhance degradability of forages and stream of microbial protein from the rumen (Wallace and Newbold, 1992). In Egypt live yeast is considerd one of the cheapest feed additives that added to sheep rations to improve animal performance and rumen parameters.

Literature about the effect of live yeast culture on rumen parameters and rumen protozoal and microbial count is conflicting, therefore this study investigate the impact of adding YC *(S. cerevisiae)*at graded levels to sheep rations on nutrients digestibility, rumen fermentation parameters and total bacterial and protozoal count in the rumen.

## MATERIALS AND METHODS

The experiment was carried out at the Animal Production Research Farm, Faculty of Agriculture, Sohag University and Animal Production Department, Faculty of Agriculture, Assiut University.

***Digestibility trials:***

The digestibility trials were carried out using fifteen rams (two years old and about 50± 1.25 kg body weight). Each trial lasted for 3 weeks, the first 2 weeks were considered as a preliminary period followed byone week collection period. Animals were classified into three groups. The control group fed a basal diet consisting of roughage and concentrate mixture, but the two treated groups fed the basal diet supplemented with either 0.5 % (T1) or 1% (T2) of yeast culture to concentrate mixture. The yeast culture used in this experiment was

manufactured by F.L. Emmert., Co. USA. The animal's requirements for crude protein (CP) and total digestible nutrients (TDN) were calculated according to NRC (1985). All animals of the three groups were fed 60% of their requirements as concentrate mixture while roughage was given *ad libitum* as chopped corn stover**.** Animals had free access to water. The rams were housed individually in metabolism cages.

***Chemical analysis and digestion coefficients measurements:***

The diet samples were taken daily during the collection period and the samples were mixed at the end of each trial, ground through 1 ml screen for chemical analysis. Faeces were collected daily and 10% of its weight were taken and dried at 60-70 ºC for 24 h. The fecal samples from each animal were composited and grounded through a 1mm mill screen for subsequent chemical analysis.

The chemical analysis of feeds residual and faeces were carried out using procedures of Association of the Official Analytical Chemists (AOAC, 1990). Neutral detergent fiber (NDF), Acid detergent fiber (ADF) and Acid detergent lignin (ADL) were determined using procedures of Goering and Van Soest (1970).The hemicellulose was calculated by the difference between NDF and ADF, while the cellulose was calculated by the difference between ADF and ADL. The apparent digestion coefficients of nutrients were calculated by expressing the difference between the content of nutrient in both consumed feed and faeces as a percentage of its intake. Chemical compositions of control, YC treated diets and corn stover are shown in Table (1).

**Table 1. Chemical composition of experimental diets as dry matter basis (%)**

| Items | Concentration mixture* | | | Corn stover |
|---|---|---|---|---|
| | Control | T1 | T2 | |
| DM | 90.82 | 89.76 | 89.24 | 91.87 |
| OM | 80.24 | 79.04 | 78.42 | 83.78 |
| Ash | 10.58 | 10.72 | 10.82 | 8.09 |
| CP | 19.07 | 20.54 | 20.69 | 6.63 |
| CF | 15.02 | 14.87 | 15.42 | 32.25 |
| EE | 1.83 | 2.42 | 2.06 | 1.48 |
| NFE | 53.50 | 51.46 | 51.00 | 51.55 |
| NDF | 31.87 | 31.22 | 30.22 | 64.74 |
| ADF | 14.89 | 15.17 | 15.02 | 32.33 |
| ADL | 5.01 | 4.95 | 5.01 | 6.25 |
| Cellulose | 9.88 | 10.22 | 10.01 | 26.08 |
| Hemicellulose | 16.98 | 16.05 | 15.20 | 32.41 |

* The ingredients of concentrate mixture were: 20 % corticated cotton seed meal, 25% wheat bran, 37% yellow corn, 12% soybean meal, 3% vinasse, 2% limestone, 1% salt.

T1: 0.5 % dry yeast culture diet, T2: 1% dry yeast culture diet

DM, dry matter; OM, organic matter; CP, crude protein; CF, Crude fiber; EE, ether extract; NFE, Nitrogen free extract; NDF, Neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin

***Rumen liquor parameters:***

Rumen content samples were collected one time from each ram using a stomach tube on the next day after collection period of each digestibility trial. Samples were taken after 4hrs of feeding. Rumen liquor samples were divided into two parts, the first part filtrated through one layer of cheesecloth, which used to measure protozoal and bacterial count. However, the second one filtrates through four layers of cheesecloth. The filtrate portion was used immediately for measurement of pH using a digital pH meter and ammonia concentration according to Conway (1962) method. Few drops of saturated solution of mercuric chloride were added to the filtrate portion to stop microbial activity before they were stored for analysis (Tabana, 1994), then samples were kept frozen (-20ºC) for subsequent determination oftotalshort chain fatty acids (VFAs). Theshort chain fatty acids (VFAs) measured using gas chromatography (GC**)** analyses Carlo Erba 5000 model (Carlo Erba, Milan, Italy). The samples of VFAs measure were prepared as described by Kroismayr and Sehm (2007).The total protozoal count was conducted according to Abou El-Naga (1967). The total rumen bacterial numbers were determined using the method performed by Newbold *et al*. (1995).

***Statistical analysis:***

Statistical analysis was done according to general linear model (G.L.M) of S.A.S program (2001), version 8.2. Differences between groups for nutrient digestibility, feeding value, nitrogen balance, total bacterial and protozoal count and rumen liquor parameters were evaluated by one-way ANOVA. Duncan Multiple Range Test (Steel and Torrie, 1980)was used to test the effect of treatments. The data were presented as mean ± S.E.M. Level of significance was set at $P<0.05$. The statistical model was as follows.

$Y_{ij} = \mu + T_i + e_{ij}$

Where: $Y_{ij}$= the observation ij, μ =the overall mean, $T_i$ = the effect due to treatment i., $e_{ij}$ = the experimental error.

## RESULTS AND DISCUSSION

***Nutrient digestibility:***

The nutrients digestibilitys of treated diets in the rumen are shown in Table 2. The dry matter (DM), organic matter and hemicellulose were improved (P<0.05) for rams supplemented with 0.5 and 1% yeast culture compared with control group. Likewise, dietary supplement of 1% YC had significant (P<0.05) effect on CP, CF and NDF digestibility in rams as compared with those fed diet supplemented with 0.5 % YC or diet with no supplement(control) groups. Newbold *et al*. (2000) found that dietary yeast increased the breakdown of fiber in the rumen, which increased microbial protein production, consequently, more protein available for absorption in the small intestine. Ghazanfar *et al*. (2015) attributed the improvement in nutrients digestibility to the increase of cellulose degrading microbial biomass population inside rumen. The improved CP digestibility by YC supplementation is confirmed with the findings of Chaucheyras- Durand *et al*. (2005)who reported that live yeast decreased the rate of peptides degradation, which lowering ammonia wastage and consequently increased the amount of rumen undegraded protein available to rams. Dietary yeasts improved growing conditions for the anaerobic rumen microbes, especially the cellulolytics through scavenging available oxygen from the surfaces of freshly ingested feeds to maintain metabolic activity and decreasing redox potential in the rumen (Chaucheyras-Durand *et al*., 2008).

**Table 2. Effect of yeast culture supplement to rams rations on nutrient digestibility**

| Item | Treatment | | | P-value |
|---|---|---|---|---|
| | Control | T1 | T2 | |
| DM | 68.30$^{b}$± 0.70 | 71.83$^{a}$ ± 0.94 | 73.08$^{a}$ ± 0.92 | 0.018 |
| OM | 68.24$^{b}$ ± 1.10 | 73.00$^{a}$ ± 0.64 | 72.01$^{ab}$ ± 1.55 | 0.059 |
| CP | 68.25$^{b}$ ± 1.09 | 67.97$^{b}$ ± 0.94 | 72.78$^{a}$ ± 0.25 | 0.012 |
| CF | 48.03$^{b}$ ± 0.98 | 50.41$^{b}$ ± 0.96 | 56.21$^{a}$ ± 1.19 | 0.003 |
| EE | 85.13± 0.57 | 85.03 ± 1.50 | 84.95± 0.93 | 0.993 |
| NFE | 76.41$^{b}$ ± 0.55 | 78.41$^{ab}$ ± 0.64 | 78.97$^{a}$ ± 0.59 | 0.051 |
| NDF | 55.73$^{b}$ ± 0.16 | 63.63$^{b}$ ± 0.56 | 65.41$^{a}$ ± 0.86 | 0.001 |
| ADF | 44.94 ± 1.18 | 44.56 ± 1.28 | 46.63 ± 0.83 | 0.429 |
| ADL | 6.18 ± 0.16 | 6.11 ± 0.16 | 5.71 ± 0.39 | 0.442 |
| Cellulose | 38.76 ± 1.10 | 38.45 ± 1.42 | 40.91 ± 1.01 | 0.349 |
| Hemicellulose | 10.79$^{b}$ ± 1.24 | 19.06$^{a}$ ± 0.92 | 18.78$^{a}$ ± 0.67 | 0.001 |

$^{a, b}$ Means within the same raw in each item with different superscripts are significantly different (P<0.05).
T1: 0.5 % yeast culture, T2: 1% yeast culture of concentrate mixture.
DM, dry matter; OM, organic matter; CP, crude protein; CF, Crude fiber; EE, ether extract; NFE, Nitrogen free extract; NDF, Neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin

***Rumen parameters:***

As shown in Table 3, supplementation of yeast culture at the level of 0.5 and 1% of the concentrate diets increased (P<0.05) the pH values, concentrations of total VFAs, acetate and propionate as compared with the control diet. However, other VFAs and the ratio of acetate to propionate and rumenal concentrations of NH3-N were lower (P<0.05) for both levels of yeast culture than control one.The higher values of rumen pH in yeast treated groups may be due to yeast cells have the ability to motivate the efficiency of *Selenomonas rumenantium* and *Megasphaera elsdenii* to utilize lactic acid in the rumen (Rossi *et al*.2004) or may be *S. cerevisiae* can compete with other bacteria to utilize starch for fermentation (Lynch and Martin, 2002), leading to the inhibition of lactate accumulation in the rumen, thereby decreasing incidences of acidosis (Calsamiglia *et al*., 2012). The mean concentration of rumen ammonia nitrogen in the present study was lower in rams received two levels of YC than control one (Table 3). Similar results were found by Lu *et al*. (2016).The lowering concentration of $NH_3$-N in the rumen may be related to more incorporation of $NH_3$-N into microbial protein resulted in improvement in microbial activity (Erasmus *et al*., 1992).

The higher value of acetate and propionate production indicated an improvement of rumen energy metabolism efficiency and/or contributed to the enhanced rumenal fermentation (Malekkhahi *et al*., 2015).Tang *et al*. (2008)found that yeast product supplement affecting the growth of different species of rumen microbes and modification of VFA production and pattern. Similarly, Harrison *et al*. (1988)stated that the addition of yeast culture containing *S. cerevisiae* had decreased the acetate to propionate ratio in cow's rumen fluid.

**Table 3. Effect of live yeast cultural supplement on rumen parameters**

| Item | Treatment | | | P- value |
|---|---|---|---|---|
| | Control | T1 | T2 | |
| pH | $6.13^{b} \pm 0.01$ | $6.35^{a} \pm 0.02$ | $6.37^{a} \pm 0.02$ | 0.001 |
| Ammonia mg/L | $87.53^{a} \pm 1.09$ | $79.60^{b} \pm 0.85$ | $75.74^{c} \pm 0.82$ | 0.001 |
| Total VFAs (m mole/L) | $146.07^{b} \pm 0.95$ | $150.27^{a} \pm 1.13$ | $153.40^{a} \pm 1.16$ | 0.008 |
| Relative proportion **%** | | | | |
| Acetate | $61.94^{b} \pm 1.09$ | $64.30^{a} \pm 0.49$ | $63.78^{a} \pm 0.32$ | 0.007 |
| Propionate | $14.06^{b} \pm 0.08$ | $16.57^{a} \pm 0.29$ | $16.34^{a} \pm 0.09$ | 0.001 |
| n-Butyrate | $17.17^{a} \pm 0.18$ | $14.13^{c} \pm 0.48$ | $15.49^{b} \pm 0.14$ | 0.001 |
| Iso-Butyrate | $2.59^{a} \pm 0.03$ | $1.57^{b} \pm 0.12$ | $1.46^{b} \pm 0.08$ | 0.001 |
| n-Valerate | $1.77^{a} \pm 0.03$ | $1.23^{c} \pm 0.03$ | $1.52^{b} \pm 0.07$ | 0.001 |
| iso-Valerate | $2.43^{a} \pm 0.05$ | $1.90^{ab} \pm 0.30$ | $1.40^{b} \pm 0.05$ | 0.017 |
| Acetate/ Propionate | $4.40^{a} \pm 0.02$ | $3.88^{b} \pm 0.07$ | $3.90^{b} \pm 0.07$ | 0.001 |

[a, b c], Means within the same raw in each item with different superscripts are significantly different (P<0.05).
T1: 0.5 % yeast culture, T2: 1% yeast culture

***Feeding value and nitrogen balance:***

As clear in Table 4, the feeding values in terms of total digestible nutrients (TDN), starch value (SV) and digestible crude protein (DCP) as well as the N retained of diets supplemented with 0.5 or 1% yeast culture were higher (P<0.05) than those of control diet. Furthermore, the DCP value of 1% YC supplement had higher (P<0.05) than DCP that of 0.5% YC treated one (Table 4).

Results in Table (4) show that the N retention ofT2washigher (P<0.05) than that of control group. However, no differences were found between T1 and T2 groups. Similar result was reported by Cole *et al*. (1992) and Malekkhahi *et al*. (2015). The higher retention of N in YC supplemented rams in the present study could explain the reduction of rumenal $NH_3$-N concentration (Table3) due to increased N incorporation into microbial protein as a result of improved microbial activity (Paryad and Rashidi, 2009).

**Table 4. Effect of yeast culture supplementation on feeding value, total microbial population and nitrogen balance**

| Item | Treatment | | | P-Value |
|---|---|---|---|---|
| | Control | T1 | T2 | |
| TDN | $60.20^{b} \pm 0.48$ | $66.40^{a} \pm 0.61$ | $66.63^{a} \pm 0.78$ | 0.001 |
| SV | $54.51^{b} \pm 0.36$ | $60.94^{a} \pm 1.48$ | $62.14^{a} \pm 1.29$ | 0.005 |
| DCP | $11.33^{c} \pm 0.60$ | $13.88^{b} \pm 0.71$ | $16.19^{a} \pm 0.65$ | 0.005 |
| Total viable bacteria, x $10^{8}$/mL | $1.66^{b} \pm 0.04$ | $2.45^{a} \pm 0.18$ | $2.72^{a} \pm 0.09$ | 0.002 |
| Total Protozoa, x 1 $0^{6}$ | $3.07^{b} \pm 0.10$ | $4.00^{a} \pm 0.11$ | $4.07^{a} \pm 0.10$ | 0.001 |
| N intake (g/day) | $16.03^{b} \pm 0.77$ | $18.77^{a} \pm 0.14$ | $19.26^{a} \pm 0.51$ | 0.011 |
| Fecal N (g/day) | 5.03 ±0.26 | 5.55 ± 0.32 | 5.33 ± 0.35 | 0.542 |
| Urinary N (g/day) | 4.71 ± 0.23 | 5.37 ± 0.54 | 4.87 ± 0.43 | 0.546 |
| Retained N (g/day) | $6.28^{b} \pm 0.94$ | $7.85^{ab} \pm 0.22$ | $9.06^{a} \pm 0.43$ | 0.049 |

[a, b] Means within the same raw in each item with different superscripts are significantly different (P<0.05).
T1: 0.5 % yeast culture, T2: 1% yeast culture

***Rumen microbial population:***

The data presented in table (4) show that the total bacterial and protozoal count were higher (P<0.05) in the rumen of YC supplemented animals than those in the rumen of control one. However, such differences in supplement YC groups were not significant. The elevated number of bacteria and protozoa in the rumen of YC supplemented animals may be due to the yeast cells contains several vitamins, enzymes, organic acids and some unknown cofactors that may enhance growth of rumen microbes (Chaucheyras *et al*.,1995). The total count of rumen cellulolytic bacteria was increased with YC addition, particularly in high concentrate diets, causing more improvement on rumen fermentation and nutrient digestibility (Chaucheyras-Durand and Fonty, 2002). Similarly, Chaucheyras-Durand *et al.* (2005)reported that the number of cellulolytic bacteria and protozoa increased in newborn lambs supplemented with yeast culture by using stomach tube.

## CONCLUSION

Supplement dietary live yeast culture (*S. cerevisiae*) particularly 1% of concentrate mixture to sheep diets may improve nutrients digestibility,

fermentation patterns, rumen microbial population and protozoal count.

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## تأثير إضافة الخميرة الحية (*Saccharomyces crevisiae*) على هضم العناصر الغذائية، تخمرات الكرش وعدد الكائنات الحية الدقيقة فى كرش الأغنام

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أجريت هذه الدراسة بغرض دراسة تأثير اضافة الخميرة على هضم العناصر الغذائية وتخمرات الكرش فى الاغنام . اجريت ٣ تجارب هضم باستخدام ٥ كباش من الأغنام لكل تجربة. كل تجربة مدتها ٣ اسابيع الـ ٢ اسبوع الاولى فترة تمهيدية يعقبها اسبوع فترة الجمع. وكانت المعاملات كما يلى : المعاملة الأولى هى مجموعة المقارنة وفيها تتغذى الأغنام على العليقة الاساسية ،المعاملة الثانية تم تغذيتها على العليقة الاساسية بالاضافة الى ٠,٥ % خميرة مضافة الى المخلوط المركز أما المعاملة الثالثة تم تغذيتها على العليقة الاساسية بالاضافة الى ١% خميرة مضافة الى المخلوط المركز. وفى جميع المعاملات تم تغطية ٦٠% من احتياجاتها من المخلوط المركز بينما تم تغذيتها على أحطاب الذرة الشامية المجروشة كمادة خشنة حتى الشبع. اوضحت النتائج ان هضم المادة الجافة ، المادة العضوية، البروتين الخام ، الالياف الخام، الالياف المقاومة للحموضة والهيموسليلوز تحسن معنويا فى مجموعات الكباش المضاف اليها الخميرة بنسبه٠,٥ و ١% مقارنة بمجموعة المقارنة. اضافة الخميرة بنسبه٠,٥ و ١% فى علائق الكباش أدى الى زيادة معنوية لقيمة رقم الحموضة فى الكرش والاحماض الدهنيه الطيارة الكليه ونسبه الخليك : البروبيونيك فى حين انخفض تركيز الامونيا فى الكرش انخفاضا معنويا مقارنة بمجموعة المقارنة.كان النيتروجين المحتجز فى الكباش المغذاة على المستويين من الخميرة اعلى معنويا مقارنهبكباش مجموعة المقارنة. اضافة الخميرة الى علائق الكباش حسنت معنويا القيمة الغذائية للعلائق مقارنة بمحموعة المقارنه. كان عدد كل من البكتريا والبروتوزوا أكبر معنويا فى المجموعات المضاف لها الخميرة مقارنة بمجموعة المقارنة. توضح هذة النتائج أن اضافة الخميرة الحية خاصة بمستوى ١% الى المخلوط المركز أدت الى تحسن النسبه الهضميه ، خصائص التخمر واعداد الكائنات الحيه الدقيقة فى كرش اغنام التجربة.