

## The Effects of Live Yeast Culture (*Saccharomyces cerevisiae*) on Rumen Fermentation and Nutrient Degradability in Yearling Lambs

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### Summary

This study was carried out in two experiments. Experiment 1 was conducted with six ruminally-cannulated one-year old Kangal Akkaraman male lambs, using a crossover design with 2 periods to determine the effects of live yeast culture supplementation on rumen fermentation. Yearlings were either supplemented with 4 g/d of *Saccharomyces cerevisiae* (BeneSacc,  $4 \times 10^9$  CFU/g) or not supplemented (control). Animals were penned individually, and were fed a diet composed of 25% forage and 75% concentrate for sixteen days. Rumen fluid was sampled on day 16, 0 h (before feeding), 3 h and 6 h after feeding. Rumen pH, the numbers of protozoa, ammonia-N and volatile fatty acids (VFA) were determined in samples. Molar proportions of acetate were decreased, and propionate was increased with supplementation of live yeast culture. No differences were observed for ruminal pH, protozoa population, total VFA and ammonia-N concentrations, between treatments. In Experiment 2, *in situ* nutrient degradability of barley grain, corn dried distillers grains with solubles (DDGS) and wheat straw were determined. Live yeast culture supplementation did not affect dry matter (DM) degradability of barley, DDGS or straw, crude protein (CP) degradability of DDGS, nor neutral detergent fiber (NDF) degradability of straw. But, it reduced the potential degradability of DDGS in rumen.

**Keywords:** Yeast, Yearling Lamb, Rumen fermentation, Degradability

## Canlı Maya Kültürünün (*Saccharomyces cerevisiae*) Toklularda Rumen Fermentasyonu ve Besin Madde Yıkılabilirliği Üzerine Etkileri

### Özet

Bu çalışma 2 deneme şeklinde yürütüldü. Deneme 1, canlı maya kültürü ilavesinin rumen fermentasyonu üzerine etkilerini tespit etmek için, rumen kanülü yerleştirilmiş 6 baş bir yaşlı Kangal Akkaraman erkek toklu ile çapraz deney düzenine göre iki dönem halinde yürütüldü. Grubun biri kontrol olarak tutulurken, diğerinin yemlerine günlük 4 g *Saccharomyces cerevisiae* içeren canlı maya (BeneSacc,  $KOB=4 \times 10^9$ ) kültürü ilave edildi. Hayvanlar bireysel bölmelerde tutuldu ve %25'i yonca, %75'i kesif yemden oluşan rasyonlarla 16 gün süreyle beslendi. 16. günde hayvanlardan yeme öncesi, yemlemeden 3 ve 6 saat sonra rumen sıvısı örnekleri alındı. Örneklerde pH, protozoon sayısı, amonyak N'u ve uçucu yağ asitleri (UYA) belirlendi. Canlı maya kültürünün ilavesi ile asetik asit oranı azaldı, propiyonik asit oranı arttı. Ruminal pH, protozoon konsantrasyonu, toplam UYA'leri ve amonyak N'u, bakımından muameleler arasında farklılık gözlenmedi. İkinci denemede; arpa, kurutulmuş mısır damıtma ürünü (KMDÜ) ve samanın *in situ* besin madde yıkılabilirlikleri tespit edildi. Canlı maya kültürü ilavesi arpa, KMDÜ, samanın KM yıkılabilirliğini, KMDÜ'nün ham protein, samanın NDF yıkılabilirliğini etkilemedi. Fakat KMDÜ'nün rumende potansiyel yıkılabilirliğini düşürdü.


**Anahtar sözcükler:** Maya, Toklu, Rumen fermentasyonu, Yıkılabilirlik


### INTRODUCTION

In the past two decades, the potential roles of specific microbial supplements have been better defined and there has been considerable interest in

using products containing living microorganisms as feed supplements for ruminants. After the use of antibiotics as growth promoters has been banned in

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2006 in European Union, the interest on yeast cultures has increased on the rumen function and microbial population. The effect of yeast cultures in animal production has been well documented mainly in dairy and meat production.

Effectiveness of yeast has been variable and strongly influenced by ration composition and yeast strain. Data indicate that supplementation of yeast in the ruminant diet may improve feed intake, fiber digestion, numbers of anaerobic and cellulolytic bacteria, and ruminal pH value. It may also alter the composition of volatile fatty acids, reduce lactate accumulation and the concentration of oxygen in the rumen fluid, and improve utilization of starch <sup>1,2</sup>. Also, yeast cultures have biologically valuable proteins combined with high potency vitamin B-complex, important trace minerals and protein <sup>3</sup>.

Products containing *Saccharomyces cerevisiae* vary widely in efficiency, primarily because of differences in strain, the viability of yeast cells, and their dosage. Doležal et al.<sup>2</sup> reported that protozoal count and VFA concentration increased with depending on increased of yeast dosage. Arcos-Garcia et al.<sup>1</sup> obtained different results from two different yeast cultures. Supplementation of the mixed yeast culture had no effect on intakes of digestible crude protein (CP) and metabolizable energy, nutrient digestibility, nitrogen balance or rumen fermentation characteristics such as pH, ammonia, VFA concentration and protozoal count <sup>4</sup>. Galip <sup>5</sup> reported that total VFA, ammonia-N concentrations and protozoal counts in the ruminal fluid were not affected by yeast culture. Similarly, Aydın et al.<sup>6</sup> found that rations with high forages or high concentrate supplemented with *Saccharomyces cerevisiae* live yeast culture had no significant effects on ruminal parameters except for the percentage of protozoa.

The purpose of the present study was to determine the effect of a live yeast culture on rumen fermentation and nutrient degradability of some feedstuffs (barley grain as an energy source, corn DDGS as a protein source, wheat straw as roughage) in yearling lambs.

## MATERIAL and METHODS

Two experiments were conducted at the Faculty of Veterinary Medicine in Selcuk University. Six yearlings Kangal Akkaraman male lambs, 60 kg body weight, fitted with permanent rumen cannulae were used. Yearlings were housed in 1x1 m individual pens with wooden screened floors. The ethical committee approval was obtained to perform of this study (SUVFEK-2009/032).

### Experiment 1

In Experiment 1, the effects of live yeast culture supplementation on rumen fermentation were investigated. The yearling lambs were fed a basal diet with 25% pelleted alfalfa and 75% concentrate, and their ration was formulated to meet 1.15 times their maintenance nutrient requirements <sup>7</sup>. Each animal was fed daily 375 g pelleted alfalfa and 1125 g commercial lamb fattening concentrate at 9.00. Three yearling lambs receiving the same basal ration once daily were supplemented with 4 g/d of live yeast culture containing *Saccharomyces cerevisiae* (BeneSacc, 4x10<sup>9</sup> CFU/g) for a period of 16 days following a cross-over design. Yearlings had free access to fresh water.

On day 16, approximately 250 ml of rumen contents were collected from the rumen of each yearling lamb at 0 h (before feeding), 3 h and 6 h after feeding. The rumen contents were squeezed through two layers of cheesecloth, and pH values of the rumen fluid were measured immediately with a digital pH meter (Hanna, HI 8314). To count protozoa, 1 ml of ruminal fluid was diluted with a 50 ml protozoa dilution solution (150 of ml pure glycerin plus 20 ml of 37% formaldehyde plus 820 ml of distilled water), stained with Brilliant green, stored at 10°C and counted using McMaster counting chamber per milliliter of ruminal fluid <sup>8</sup>. Two 10 ml of ruminal fluid samples were acidified with 2-3 drops of concentrated sulphuric acid for measuring ammonia-N and 2 ml of 25% metaphosphoric acid for determining of VFA <sup>9</sup>, and stored in a freezer at -20°C until analyzing. Ammonia-N was measured via spectrophotometer (Shimadzu UVmini-1240) according to the method of Chaney and Marbach <sup>10</sup>. Volatile fatty acids were determined by Shimadzu 15A gas chromatography <sup>9</sup>. Some nutrients of forage and concentrate fed to yearlings were analyzed according to the methods of AOAC <sup>13</sup> (Table 1).

Data in experiment 1 were analyzed using the GLM procedure of Minitab 15 <sup>11</sup> according to a Cross-over design with the model:

$$Y_{ijk} = \mu + seq_i + lamb_{ij} + per_k + trt_h + e_{ijk}$$

Where  $Y_{ijk}$  = the data during the  $k^{th}$  period of the  $j^{th}$  lamb in the  $i^{th}$  group ( $i=1,2$ ;  $j=1-3$ ;  $k=1,2$ )

$\mu$  = the overall mean effect

$seq_i$  = the effect of the  $i^{th}$  sequence group ( $i = 1,2$ )

$lamb_{ij}$  = the effect of the  $j^{th}$  lamb on the  $i^{th}$  sequence ( $j = 1-3$ )

$per_k$  = the effect of the  $k^{th}$  period ( $k = 1,2$ )

$trt_h$  = the effect of the  $h^{th}$  treatment ( $h = 1,2$ ; being a function of  $i$  and  $k$ )

$e_{ijk}$  = the random error

**Table 1.** Chemical composition of feeds offered to yearlings, g/kg DM**Tablo 1.** Toklulara verilen yemlerin kimyasal bileşimleri, g/kg KM

Feeds	DM	Ash	EE	CP	NDF	ADF
Pelleted alfalfa	913.5	127.3	30.8	158.2	502.9	451.3
Concentrate *	918.2	67.6	59.6	170.4	394.6	157.5

\*: Barley 10%, corn 18%, sunflower meal 20%, wheat bran 20%, horse bean 10%, corn gluten meal 18.4%, limestone 2.5%, salt 1%, vitamin-mineral premix 0.1% (per kg: 1.300.000 IU of vitamin A, 260.000 IU of vitamin D<sub>3</sub>, 3.000 mg of vitamin E, 120.000 mg of niacin, 5.000 mg of Mn, 5.000 mg of Fe, 5.000 mg of Zn, 1.000 mg of Cu, 15 mg of Co, 80 mg of I, 15 mg of Se, 18.000 mg of P, 300.000 mg of Ca)

### Experiment 2

Experiment 2 was conducted with same animals that were used in Experiment 1. The effects of 4 g/day live yeast culture supplementation were tested on nutrient degradability of barley, corn DDGS and wheat straw, as energy, protein and fiber sources, respectively. Feed samples were ground and passed through a 2-mm sieve for *in situ* degradability. The *in situ* nutrient degradability was determined<sup>12</sup> by incubating air dry samples of 4-5 g barley or corn DDGS and 2.5 g wheat straw in triplicate nylon bags (5x11 cm; 45-µm pore size).

In order to determine the DM degradability from the nylon bag and degradability characteristics of the feed, the samples were incubated in the rumen and withdrawn after the following times: 2, 4, 6, 12, 24 h for barley; 2, 4, 6, 12, 24, 48 h for corn DDGS; and 6, 12, 24, 48, 96 h for wheat straw. After the removal of bags, the bags were immediately rinsed in tap water to remove the debris and stop fermentation. All bags were washed under tap water for approximately 30 min, until the water remained clear. To determine the undegraded DM, the bags were dried for 48 h at 65°C. Washing loss was determined by similarly washing triplicate bags containing samples that were not incubated in the rumen. Triplicate bags were dried in the same way to determine the DM content of the samples<sup>12</sup>. Bags were weighed and corn DDGS residues analyzed for CP<sup>13</sup> and wheat straw residues analyzed for NDF<sup>14</sup>. The DM, ash, and CP concentrations for barley and corn DDGS, as well as the NDF concentrations for wheat straw were also analyzed similarly (Table 3).

Results from the *in situ* study were fitted to the exponential model  $p=a+b(1-e^{-ct})$  as described by McDonald<sup>15</sup> using the Neway programme, where: p=DM degradability at time t; a=soluble fraction; b=insoluble but fermentable fraction; c=rate of degradation of B; t=time incubation; A=washing loss (readily soluble fraction); B=(a+b)-A; potential degradation (PD)=(A+B);

effective degradability (ED)= $A+[Bc/(c+k)]$  at rumen outflow rates (k) of 0.05 h<sup>-1</sup>.

Data in Experiment 2 were analyzed by t-test<sup>16</sup>.

## RESULTS

### Experiment 1

Ruminal pH, ammonia-N and total protozoal count were not affected by live yeast culture supplementation at 4 g/day (Table 2).

There were no statistically significant effects of live yeast culture supplementation on total VFA. Molar proportion of acetate in control yearlings was higher (P<0.05) than in yearlings supplemented with live yeast culture at 0 h (before feeding) and 6 h after feeding. On the contrary, molar proportion of propionate was higher at the same sampling times with live yeast culture supplementation (P<0.05) (Table 2).

**Table 2.** Effects of yeast supplementation on ruminal pH, ammonia-N (mg/l), protozoal count (x10<sup>4</sup>/ml), total volatile fatty acids (mmol/l) and their molar proportions at different sampling times (n=3)**Tablo 2.** Maya ilavesinin farklı örnekleme zamanlarında rumen sıvısı pH'si, amonyak-N'u (mg/l) protozoon sayısı (x10<sup>4</sup>/ml), toplam uçucu yağ asitleri (mmol/l) ve oranlarına etkisi (n=3)

Item	Control	Yeast	SEM	P
<b>pH</b>				
0 h	6.91	6.81	0.09	0.474
3 h	5.61	5.41	0.10	0.213
6 h	5.64	5.47	0.05	0.080
<b>Ammonia-N</b>				
0 h	112.74	105.70	13.45	0.727
3 h	137.50	140.39	11.30	0.864
6 h	121.44	124.55	22.11	0.925
<b>Total Protozoal Count</b>				
0 h	31.13	16.15	10.30	0.353
3 h	8.36	9.44	4.48	0.872
6 h	5.70	9.29	3.76	0.529
<b>Total Volatile Fatty Acids</b>				
0 h	42.21	45.79	4.86	0.624
3 h	136.54	151.09	8.44	0.277
6 h	100.56	124.33	8.79	0.114
<b>Acetate</b>				
0 h	62.58	55.52	0.57	0.000
3 h	58.97	54.19	1.71	0.105
6 h	58.29	52.77	1.17	0.021
<b>Propionate</b>				
0 h	17.61	22.84	0.20	0.000
3 h	18.56	21.07	1.32	0.238
6 h	16.62	21.47	0.81	0.008

### Experiment 2

As seen in [Table 4](#), live yeast culture supplementation increased the soluble fraction (a), but decreased the insoluble but fermentable fraction (b) and degradation

**Table 3.** Chemical composition of barley grain, corn DDGS and wheat straw, %

**Table 3.** Arpa, KMDÜ ve buğday samanının kimyasal bileşimleri, %

Feedstuff	DM	Ash	CP	NDF
Barley	92.06	2.22	11.42	-
Corn DDGS	93.83	4.62	24.89	-
Wheat straw	94.00	10.47	2.22	75.64

**Table 4.** Dry matter degradability characteristics of barley grain, corn DDGS and wheat straw, % (n=9)

**Table 4.** Arpa, KMDÜ ve buğday samanının KM yıkılabilirlik özellikleri, % (n=9)

Feeds		a		b		c		ED (0.05)		PD	
		Control	Yeast	Control	Yeast	Control	Yeast	Control	Yeast	Control	Yeast
Barley Grain	X	52.81	63.39	25.51	16.88	0.370	0.128	80.20	85.56	78.32	80.27
	Sx	4.91	1.06	3.58	1.16	0.103	0.010	3.94	2.56	1.57	2.17
	P	0.042		0.030		0.025		0.263		0.491	
Corn DDGS	X	53.23	51.12	33.94	24.51	0.025	0.061	66.18	64.88	87.17	75.63
	Sx	0.87	0.44	2.58	0.32	0.003	0.007	1.32	0.95	1.82	0.46
	P	0.048		0.003		0.000		0.437		0.000	
Wheat Straw	X	7.01	6.14	34.89	34.85	0.019	0.029	16.56	18.44	41.89	40.99
	Sx	0.61	0.90	2.99	4.47	0.003	0.004	0.78	1.31	3.48	5.25
	P	0.439		0.994		0.045		0.238		0.889	

Washing losses, %: barley grain: 30.58; corn DDGS: 45.00; wheat straw: 10.78

**a:** soluble fraction; **b:** insoluble but fermentable fraction; **c:** the rate of degradation of b; **ED(0.05):** effective degradability at rumen outflow rates of 0.05 h<sup>-1</sup>; **PD:** potential degradability

**Table 5.** Effects of yeast supplementation on CP degradability of corn DDGS, and NDF degradability (kg/kg) of wheat straw at different incubation times, (n=3)

**Table 5.** Maya ilavesinin farklı inkübasyon sürelerinde KMDÜ'nün HP, buğday samanının NDF yıkılabilirliği (kg/kg) üzerine etkileri, (n=3)

Inc.time, h	Control		Yeast	
	X	Sx	X	Sx
<b>CP Degradability of Corn DDGS</b>				
2	0.712	0.028	0.711	0.022
4	0.782	0.027	0.761	0.025
6	0.770	0.010	0.755	0.029
12	0.782	0.014	0.797	0.001
24	0.904	0.013	0.918	0.027
48	0.972	0.014	0.938	0.016
<b>NDF Degradability of Wheat Straw</b>				
6	0.109	0.008	0.120	0.025
12	0.179	0.011	0.172	0.001
24	0.204	0.002	0.243	0.022
48	0.291	0.015	0.269	0.011
96	0.380	0.040	0.380	0.072

rate (c) of barley (P<0.05). Soluble (P<0.05) and b (P<0.01) fractions of corn DDGS were lower with live yeast culture supplementation, while c value was higher (P<0.001) with live yeast culture supplementation compared to control. Live yeast culture supplementation decreased the potential degradability of corn DDGS (P<0.001). The c value of wheat straw increased with live yeast culture supplementation (P<0.05), but unchanged a, b, ED and PD values.

The rate of *in situ* CP degradability of corn DDGS, and NDF degradability of wheat straw ([Table 5](#)) were not different between treatments.

## DISCUSSION

### Experiment 1

Although ruminal pH was not affected statistically with live yeast culture supplementation, live yeast culture supplementation did result in numerically decrease rumen pH comparing with the control at all sampling times. There was no consistent previous reports on yeast supplementation on the rumen pH values. In this reports, ruminal pH was found to be decrease <sup>1</sup>, unchanged <sup>4,6,17,18</sup> and increase <sup>5,19</sup>.

Similar to this experiment, most other studies found that microbial cultures based on *Saccharomyces cerevisiae* had no effect on ruminal ammonia-N <sup>4,5,17</sup>. However, some studies found decrease <sup>18</sup> or increase <sup>20</sup>. The response could be associated with the characteristics of the diet.

Yeast culture supplementation in the current experiment did not have any effect on protozoal number

as also observed by Mwenya et al.<sup>17</sup>, Galip<sup>5</sup>, and Tripathi et al.<sup>4</sup>.

Concentration of total VFA with live yeast culture supplementation was similar to control, but molar proportion of acetate decreased, and propionate increased ( $P < 0.05$ ) (Table 2). The quick microbial fermentation increased total VFA concentration sharply related to high concentrate in the diet or single meal feeding. Some researchers<sup>1,18</sup> found increases in total VFA, while Thrune et al.<sup>19</sup> reported a decrease when yeast culture was added to the diet. Acetate production is mainly due to the fermentation of structural carbohydrates by cellulolytic bacteria while propionate production is mainly due to the fermentation of non-structural carbohydrates by amylolytic bacteria. The addition of yeast cultures can stimulate amylolytic bacteria that would use preferably true degradable protein. Increased molar proportion of propionate can help meat or milk production being more efficient. Similar to the current results, Lascano and Heinrichs<sup>18</sup> reported that yeast supplementation decreased acetate and increased propionate. On the contrary, Mwenya et al.<sup>17</sup> found that acetate increased and propionate decreased with yeast culture supplementation.

### Experiment 2

In the current study, the washing loss of barley grain was 30.58%, which was similar to report of Woods et al.<sup>22</sup>, but higher than of Taghizadeh and Zabihollah<sup>21</sup> and lower than of Afzalzadeh et al.<sup>23</sup>. There was no other research on the effect of yeast supplementation on DM degradation of barley. In this experiment, live yeast culture supplementation increased soluble fraction (a), while decreasing fermentable fraction (b) and degradation rate (c) of barley ( $P < 0.05$ ) (Table 4).

Woods et al.<sup>22</sup> and Afzalzadeh et al.<sup>23</sup> reported similar soluble fraction for barley as in the current research. Fermentable fraction (b) in the present study was similar to the finding of Afzalzadeh et al.<sup>23</sup>, but lower than findings of Taghizadeh and Zabihollah<sup>21</sup> and Nikkhah et al.<sup>24</sup>. Effective degradability of barley DM at rumen outflow rates of  $0.05 \text{ h}^{-1}$  (ED 0.05) in control and yeast were 80.20 and 85.56%, respectively. This observation is in accordance with some reports<sup>22,23</sup>, but higher than other<sup>21</sup>. Nikkhah et al.<sup>24</sup> reported the potential degradability as 79.4, 77.8 or 68.1% for 3 barley cultivars ground through 2, 4 or 6 mm screens. In the current study, barley was ground at 2-mm sieve and potential degradation values were very similar (78.32 with control and 80.27 with live yeast culture) to the finding of Nikkhah et al.<sup>24</sup> and lower than of Afzalzadeh et al.<sup>23</sup>.

No published report about the effect of yeast supplementation on DM degradation of corn DDGS was found. The soluble fraction, insoluble but fermentable fraction, degradation rate, and the potential degradability of corn DDGS were greater in control than yeast culture supplementation ( $P < 0.05$ ) (Table 4). The decrease of potential degradability and the increase of propionate (experiment 1, Table 2) with yeast culture supplementation could explain why live yeast had a positive effect on the microbial protein production. Notwithstanding, the CP degradability of corn DDGS was not affected by the addition of live yeast culture (Table 5).

Washing loss of corn DDGS was 45.00%. In one of the few published data dealing with *in situ* DM degradability of corn DDGS, Woods et al.<sup>22</sup> reported lower washing loss, effective degradability, and potential degradation than in the current research. In the current research, the degradation rate was 0.025 and 0.061 for control and live yeast culture, respectively, which is similar to Woods et al.<sup>22</sup>.

Live yeast culture supplementation increased the degradation rate of b ( $P < 0.05$ ), but had no effect on soluble fraction, insoluble but fermentable fraction, effective degradability, and potential degradation of wheat straw (Table 4). In the present study, soluble fraction of straw was lower than one reported by Jalilvand et al.<sup>25</sup>. Also, insoluble but fermentable fraction was similar to that of this reported. Jalilvand et al.<sup>25</sup> reported higher effective degradability with wheat straw than in this current study.

Olson et al.<sup>26</sup> reported that *in situ* NDF degradability of mixed-grass improved with the addition of yeast, whereas no difference could be found in the current research (Table 5). Likewise, Corona et al.<sup>27</sup> observed that yeast supplementation was not affected *in situ* NDF degradability or NDF digestibility. Fadel Elseed and Abusamra<sup>28</sup> reported yeast supplementation increased OM digestibility by 10.1% and NDF digestibility by 10.5% for 5 g/day yeast supplementation in goat's kids ration. Arcos-Garcia et al.<sup>1</sup> reported that forage quality may affect the response to yeast culture in NDF digestion, and that more benefits can be obtained with good quality roughages.

As a conclusion, a commercial live yeast culture addition to the diets of yearling lambs at 4 g/day did not affect ruminal pH, total protozoal number, total VFA and ammonia-N concentration. The molar proportion of acetate was decreased while propionate was increased by the addition of live yeast culture. *In situ* CP degradability of corn DDGS or NDF degradability of wheat straw was not changed by yeast culture supplementation.



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