

Effect of Yeast Culture as an Additive to Sheep Feed on Performance, Digestibility, Nitrogen Balance and Rumen Fermentation

B.M. Ahmed and M. S. Salah

Department of Animal Production and Breeding, College of Agriculture and Veterinary Medicine, King Saud University, Gassim, Buriyadah, P. O. Box. 1482, Saudi Arabia

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Abstract. The effect of using different levels of yeast culture as a feed additive to sheep rations was investigated in two trials; the 1st trial was to determine the nutrient digestibility, N-balance and feeding value of the experimental rations as well as rumen fermentation, using 3 fistulated rams distributed in a 3 x 3 Latin square design to 3 different rations [basal ration (control, C), control plus 4g yeast/ram/day (C4) and control plus 8g yeast/ram/day (C8)]. Each feeding period consisted of 3 weeks (2 weeks as an adaptation period prior to 1 week of sample collection). Animals were fed separately in individual metabolic cages. Rumen liquor samples were collected before (0h) and 1, 3 and 6h post morning feeding during the last 3 days of the collection period. The 2nd trial was to study the lamb performance. Twenty-four growing lambs (14.6 ± 1.3 kg) were divided into 3 comparable groups each was fed one of the above mentioned diets for 4 months. Weekly feed intake, and biweekly body weights were recorded. Growth rate and feeding efficiency were calculated. Results revealed that the addition of yeast improved the digestibility of DM, CP and CF leading to an increase in the nutritive value (TDN, DCP and ME). Nitrogen balance as well as the total VFA in the rumen was also improved with the addition of yeast at the two levels used, however, differences were only significant between C8 and the control group. The ADG of growing lambs was increased by 13.8 and 30.2% in groups C4 and C8, respectively over the C group, however, differences were not significant. It could be concluded that YC be used at the level of 8g/head/day in order to have better performance. More studies, however, are still needed especially with small ruminants (sheep and goats) to throw more lights on the effect of YC and its mode of action.

Introduction

In the recent years, the use of feed additives containing bacterial and yeast cultures (YC) has been increased. These probiotics are live microbial feed supplements, which beneficially affect the host animal by improving its intestinal microbial balance [1]. They have been used as growth promoters to replace the widely used antibiotic and synthetic chemical feed supplements [2-5]. *Lactobacilli* and *streptococci* are the most commonly used genera of microorganisms in the production of probiotics [6, 7]. *Saccharomyces cerevisiae* (as live YC) reported to balance the energy and the acid-base metabolism in

dairy cattle, resulted in a significantly higher milk production [3]. No literature, however, are available about the effect of YC on the growth performance of sheep and/or goats.

An increase in bacterial numbers recovered from the rumen is the most reproducible effect of dietary yeast supplementation, and it has been suggested that this increase is central to the action of the yeast in improving ruminant productivity (feed efficiency, milk production and composition) [8, p.317-353]. Yeast has been shown to provide nutrients that stimulate the growth of certain rumen microorganisms (probiotic effect) such as the lactic acid-utilizing rumen bacterium *Selenomonas ruminantium* [9, 10]. *S. cerevisiae* stimulation of rumen bacteria depends on its respiratory activity [11] which allow it to scavenge O₂, thus protecting the strictly anaerobic bacteria [12]. Its content of malic acid [9, 10] has little to do with its action. Yeast also provides vitamins to support the growth of rumen fungi [13]. The presence of respiring yeast, therefore, would be predicted to be beneficial to the rumen microflora. Yeast culture was reported to have positive effects on nutrient digestibility and rumen activity [14,15] while it had no effects on other studies [16,17]. The present work was carried out to study the effect of adding YC at graded levels to sheep rations on their feed utilization and productive performance. Rumen fermentation parameters were also determined in order to clarify some of the yeast mode of action.

Materials and Methods

Two experiments were carried out in order to determine the efficiency of yeast-supplemented rations in feeding sheep.

Experiment 1: Digestibility, N-balance and rumen fermentation:

Three Naemy (local Saudi Arabian breed) rams (40 kg avg. BW) in a 3x3 Latin-square design were used in a digestibility trial to determine the nutrient digestibility, N-balance, feeding value and rumen fermentation parameters of the experimental rations. The basal ration (control, C) contained a mixture of barley, 25%; wheat bran, 25% and good quality chopped Rhodes grass hay, 50%. The chemical composition of these ingredients is presented in Table 1. Treatments consisted of a YC-free basal ration (C), and the basal ration supplemented with either 4g (C4) or 8g (C8) of YC/ram/day. Animal's requirements were met according to Church [18, p. 209-222]. Yeast culture (Yea-Sacc, product of Alltech Biotechnology Center, Kentucky, USA) was included in the ration by simple mixing with wheat bran just before the morning feeding. Animals were kept and fed in individual metabolic cages allowing a separate collection of feces and urine as described by Maynard *et al.* [19, p.283-338]. Animals were fed twice daily and water was available at all times. Animals were adapted to the rations and cages for two weeks as a preliminary period (during which the exact feed intake was determined) followed by a collection period of one week (during which the feed offered was restricted to only 95% of the exact feed intake during the preliminary period to insure no refusals). During the collection week feces and urine were daily collected quantitatively and were sampled for analysis. Chemical composition of dry matter (DM), crude protein (CP), ether extract (EE), crude fiber (CF),

nitrogen-free extract (NFE) and ash for both feed and fecal samples as well as total nitrogen (N) in urine were conducted according to A.O.A.C. [20, p. 125-142].

Table 1. Chemical composition of the feed ingredients used in formulation of the basal ratio (% on DM basis)

Item	Barley	Wheat bran	Rhodes hay	Basal ratio*
Dry matter, DM	93.20	90.41	91.10	91.45
Organic matter, OM	96.94	93.89	85.51	90.50
Crude protein, CP	11.44	13.04	8.07	10.16
Ether extract, EE	2.21	3.00	1.23	1.91
N-free extract, NFE	78.76	65.90	44.22	58.38
Crude fiber, CF	4.53	11.97	32.00	20.05

* Calculated based on composition of barley, wheat bran and rhodesgrass hay.

For the rumen fermentation study, animals were fistulated prior to the experiment. Rumen liquor samples (100 ml) were collected by suction through the fistulae during the last 3 days of the collection period before the morning meal (0h) and 1, 3 and 6 h post-feeding to determine the production of volatile fatty acids (VFA) and ammonia-N concentration. Rumen digesta was strained through four layers of cheesecloth; pH was immediately measured using a hand pH-meter with glass electrode followed by the addition of 2 ml H₂SO₄ (50%v/v) to retard ammonia loss. Samples were frozen for subsequent analysis for ammonia-N according to Al-Rabbat *et al.* [21], and total VFA as described by Warner [22].

Experiment 2: Growth performance of lambs

Twenty-four growing Naemy lambs with an average body weight of 14 ± 1.5 kg were used in this experiment. Animals were divided into 3 comparable groups (8 lambs each) and randomly allocated to the three diets (**C**, **C4**, and **C8**) as described in the 1st trial. The experimental rations were formulated to meet the requirement of the animals according to Church [18, p. 209-222]. However, to have more pronounced effect of YC on microbial protein, dietary protein was minimized to the least requirements (10%). Dry YC was mixed as mentioned in experiment 1. Lambs were group-fed twice daily at 8 a.m. and 3 p.m. In the morning meal, only half of the calculated requirement was offered to the animals to insure the complete consumption of YC, while in the second meal rations were left before the animals to eat *ad lib.* in order to measure feed intake. Drinking water and mineral licks were available all the time. The amount of ration offered was changed every two weeks according to body weight changes keeping YC levels unchanged. The feeding trial lasted for 4 months during which live body weight was recorded every 2 weeks while feed intake was recorded at weekly intervals by subtracting the refusals (if any) from the amount offered. Average daily gain (ADG) and feed efficiency were calculated.

Statistical analysis

Data of the digestibility, N balance and rumen fermentation parameters were analyzed by ANOVA for Latin square design according to Gill [23, pp.132-140] using the model:

$$Y_{ijk} = \mu + P_i + A_j + T_k + e_{ijk}$$

where Y_{ijk} is the dependent variable, μ is the overall mean, P_i is the effect of i^{th} period, A_j is the effect of j^{th} ram, T_k is the effect of the k^{th} YC supplementation and e_{ijk} is the residual error assumed to be normally and independently distributed. Due to the repeated measurements of the rumen samples within each treatment, the above mentioned model was used as a split plot Latin square to analyze the data of rumen fermentation (VFA and $\text{NH}_3\text{-N}$).

Data of growth performance were analyzed by ANOVA for completely randomized design according to Gill [24, pp. 287-303] using the model:

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

where Y_{ij} is the dependent variable, μ is the overall mean, T_i is the effect of the j^{th} YC supplementation and e_{ij} is the residual error assumed to be independently and randomly distributed.

Duncan's test [25] was used to compare the treatment means.

Results

Results of experiment 1 (Table 2) revealed that the digestion coefficients of DM ($P<0.05$) and CF ($P<0.01$) were improved with animals fed the YC at both levels used in comparison with those fed YC-free diet, while difference regarding CP was only significant between control and **C8**; differences between the two levels, however, were not significant. The dietary treatment had no effect on the digestibility of EE and NFE. The increase in digestibility lead to an increase in the feeding values (Table 2). Total digestible nutrients (TDN) increased from 64.75% in the control group to 67.79 and 70% in groups fed 4 and 8g YC/head/day, respectively. Corresponding values were found for digestible crude protein (DCP) being 7.04, 7.35 and 7.66%. The improvement in TDN and DCP were only significant ($P<0.05$) for **C8** group in comparison to **C** group. The calculated metabolizable energy (ME) being 2.34, 2.45 and 2.53 Mcal/kg DM, respectively. Differences

Table 2. Digestion coefficients and nutritive value of the experimental diets as affected by the addition of yeast culture

Item	Experimental ratios ^(a)		
	Control	C 4	C 8
<i>Digestion coefficients (%)</i>			
DM	65.31 ± 0.85 ^a	70.62 ± 1.02 ^b	73.72 ± 1.07 ^b
CP	69.34 ± 0.87 ^a	72.38 ± 0.96 ^{a,b}	75.42 ± 1.19 ^b
EE	64.28 ± 1.28 ^a	65.68 ± 0.97 ^a	65.49 ± 1.16 ^a
NFE	75.47 ± 1.31 ^a	77.23 ± 1.63 ^a	79.51 ± 1.47 ^a
CF	54.28 ± 0.86 ^A	62.51 ± 0.96 ^B	65.38 ± 0.74 ^B
<i>Nutritive value</i>			
TDN, % ¹	64.75 ± 1.42 ^a	67.79 ± 1.34 ^{ab}	70.00 ± 1.53 ^b
DCP, % ²	7.04 ± 0.09 ^a	7.35 ± 0.10 ^{ab}	7.66 ± 0.12 ^b
ME (Kcal/kg DM) ³	2341	2451	2531

^{a,b} Values having different superscript within each row are significantly different ($P < 0.05$)

^{A,B} Values having different superscript within each row are significantly different ($P < 0.01$)

^a Control = basal diet; C4 = control + 4g YC/h/d; C8 = control + 8g YC/h/d

¹TDN = total digestible nutrient.

²DCP = digestible crude protein.

³ME = metabolizable energy (calculated assuming 1g TDN=3.6155 kcal; Church [18, p.313]).

Rumen ammonia-N concentration of sheep fed different levels of YC is illustrated in Fig. 1. Before feeding (at zero time) ammonia-N was almost equal in all treatments; the maximum values were found at 1 h post-feeding being a little higher with the control group. After the first hour, ammonia-N concentrations started to decline linearly to reach the minimum at 6-h post-feeding. Differences between treatments within each sampling time were not significant.

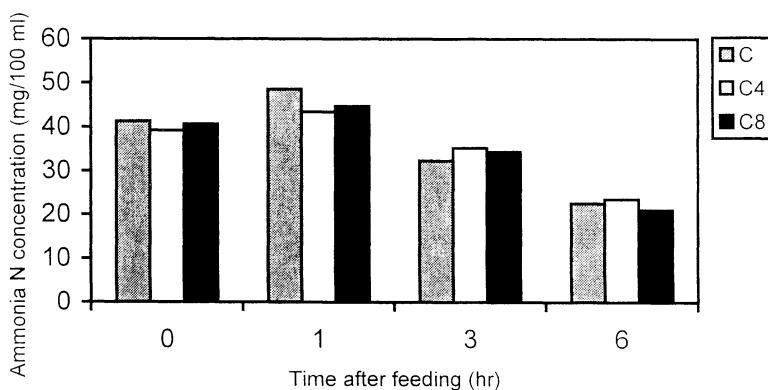


Fig. 1. Ruminal ammonial N concentration as affected by dietary yeast culture.

Concentrations of VFA as affected by the dietary YC are shown in Fig. 2. The lowest total VFA values were reported before feeding for all dietary treatments. At 1h after feeding, sheep of group **C8** (with the higher level of YC) had the highest total VFA concentration followed by those in group **C4**, whereas sheep of **C** group was the lowest one. The same trend was observed at 3 and 6h after feeding; differences between treatment within all sampling times after feeding was only significant ($P<0.05$) for group **C8** in comparison to the control group. The total VFA concentration at 3h post-feeding was the highest ($P<0.05$) of all sampling times. The increase in total VFA concentrations post-feeding lead to decreases in the pH values of rumen liquor, especially at 3h (Fig. 3). However, no significant differences in the pH values were found between treatment groups.

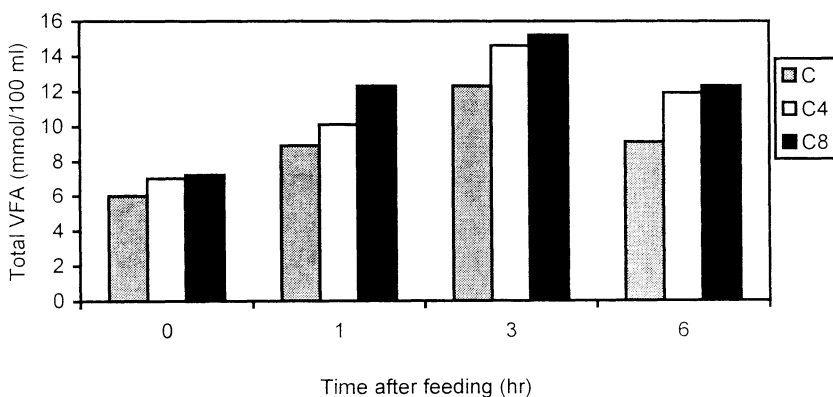


Fig. 2. Rumina total VFA production as affected by dietary yeast culture.

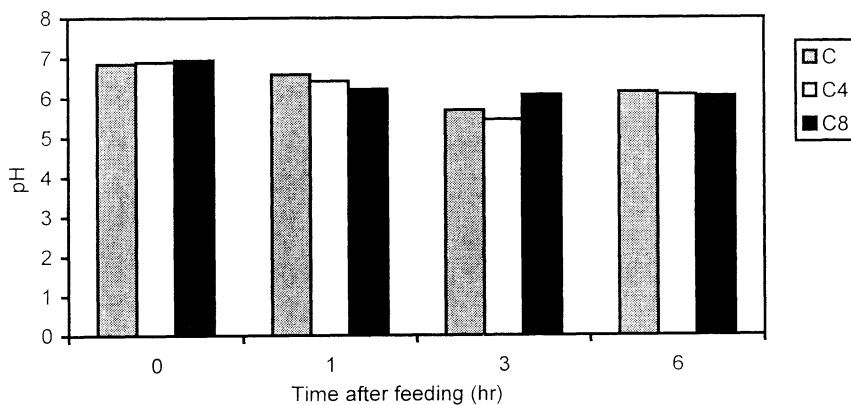


Fig. 3. Ruminal pH values as affected by dietary yeast culture.

Results of the nitrogen balance are presented in Table 3. Sheep fed YC had more, but not significant, nitrogen intake (NI). That was due to the more feed consumed in these groups. Fecal N significantly ($p<0.05$) decreased with YC-fed animals due to more digested N (DN). Urinary N was increased with YC groups. Nitrogen balance (NB) was higher ($P<0.05$) with sheep fed YC supplemented diet at the **C8** level compared to that fed YC-free diet. Group **C4** had an intermediate nitrogen balance value and did not differ significantly than the other two groups. Nitrogen balance as a percentage of NI was better with **C8** group (24.23%) followed by **C4** (22.76%) and the least was the control group (21.53%); differences were significant ($P<0.05$). However, when NB was calculated as percentage of DN, differences between dietary treatments were diminished.

Table 3. Nitrogen balance (g/head/day) of sheep as affected by dietary yeast culture

Item	Experimental rations ^a		
	Control	C 4	C 8
N intake (NI)	18.86 ± 1.23 ^a	19.38 ± 1.54 ^a	20.06 ± 1.97 ^a
Fecal N (FN)	5.78 ± 0.14 ^b	5.34 ± 0.08 ^{ab}	4.93 ± 0.10 ^a
Digested N (DN)	13.08 ± 0.16 ^a	14.03 ± 0.16 ^b	15.14 ± 0.25 ^c
Urinary N (UN)	9.02 ± 0.10 ^a	9.62 ± 0.08 ^b	10.27 ± 0.14 ^c
N balance (NB)	4.06 ± 0.04 ^a	4.41 ± 0.06 ^{ab}	4.86 ± 0.08 ^b
NB/NI, %	21.53 ± 0.25 ^a	22.76 ± 0.26 ^b	24.23 ± 0.33 ^c
NB/DN, %	31.04 ± 0.33 ^a	31.43 ± 0.30 ^a	32.10 ± 0.36 ^a

^{a,b,c} Values having different superscript within each row are significantly different ($P<0.05$)

^a Control = basal diet; C4 = control + 4g YC/h/d; C8 = control + 8g YC/h/d

The effect of dietary YC on lamb productive performance is presented in Table 4. Lambs had an average initial body weight of 14.5 kg ± 1.4. The final body weights were 29.8, 31.6 and 34.7 kg for **C**, **C4** and **C8** groups, respectively. Average daily gain was 126.7, 144.2 and 165 g/d for the same respective groups. Dietary YC improved the growth rate by 13.8 and 30.2% at the levels of 4 and 8 g/h/d, respectively over the control diet; differences however, failed to be significant. Lambs fed the treated groups consumed more feed as DM, TDN, ME, CP and DCP. Due to the group feeding system used in the present study, data of feed intake and feed efficiency were not statistically analyzed. Lambs fed the YC treated diets utilized their feed more efficiently (Table 4).

Table 4. Performance of growing Naemy lambs as affected by dietary addition of yeast culture

Item No. of Lambs	Experimental ratios ^(a)		
	Control 8	C4 8	C8 8
Avg. initial weight (kg)	14.6 ± 1.5	14.3 ± 1.2	14.9 ± 1.4
Avg. final weight (kg)	29.8 ± 2.3	31.6 ± 2.7	34.7 ± 2.5
Growth period (day)	120	120	120
Avg. total gain (kg)	15.2 ± 1.2	17.3 ± 1.5	19.8 ± 1.3
Avg. daily gain (g)	126.7 ± 19.6	144.2 ± 21.3	165.0 ± 13.5
Improvement, %	-	13.8	30.2
Feed intake/lamb/day			
DM (g)	849	890	915
TDN (g)	550	603	640
ME (Kcal)	1989	2180	2314
CP (g)	86.3	90.4	92.9
DCP (g)	59.8	65.4	70.1
Efficiency of feed utilization			
Kg DM/kg gain	6.70	6.17	5.55
Kg TDN/kg gain	4.34	4.18	3.88
PER*	1.47	1.60	1.78

*PER= protein efficiency ratio = g body weight gain/g protein consumed

^(a)Control = basal diet; C4 = control + 4g YC/h/d; C8 = control + 8g YC/h/d

Discussion

Yeast culture has been observed to improve the digestibility of most nutrients [26 - 32]; that was the case in the present study (Table 2). Robinson [26] reported that supplementation of YC in the diet increased net digestion in the forestomach, particularly of fiber leading to increase energy output. Earlier work [33] found that yeast increased the initial rate of forage digestion in the rumen. The increase in digestibility, especially for CF, may have been due to an increase in the population [11, 30] and/or activity [28, 34] of rumen cellulolytic bacteria. Proteolytic bacteria counts were also stimulated by yeast culture [35].

To clarify the mode of action of YC in the present study, ruminal microbial activity was evaluated as pH and concentrations of ammonia-N and total VFA. The concentration of total VFA was higher ($P<0.05$) at all sampling times with the group fed 8g YC in comparison to the control group. Sheep fed 4g YC had an intermediate value and did not

differ significantly than the other two groups. The control group (YC-free group) was the lowest. Total VFA was increased in all groups with the advance in time reaching the maximum activity at 3h after feeding then declined (Fig. 2). This may have been due to the increase in the bacterial counts and activity [11, 28, 30, 34, 35] and the stability of the ruminal environment [36, p 1-47]. Similar trend was reported [37]. Addition of YC to the ration had no significant effect on ammonia-N concentration or pH of the rumen fluid within all sampling times (Fig. 1&3). Others [27, 35] have reported similar results. The differences between our results and other published data [17, 27, 35] may be attributed to differences in the quantities used and/or different strains of YC. Newbold *et al.* [38] reported that some strains of yeast are effective whereas others are not. The ability of different yeast preparations to stimulate the viable count of bacteria in the sheep rumen appears to correspond with their ability to remove O₂ from rumen fluid [11]. The amount of O₂ entering the rumen of sheep daily was calculated to be in the range of 11.5 – 38 liters through saliva, food and diffusion of the blood of the host animal [11, 39]. Oxygen is known to be toxic to anaerobic bacteria and it inhibits the growth of rumen bacteria in pure culture studies [40, 41] and the adhesion of cellulolytic rumen bacteria to cellulose [42]. The presence of a respiring yeast, therefore, would be predicted to be beneficial to the rumen microorganisms.

Nitrogen balance and metabolism was found to be improved due to the inclusion of YC in the diet of sheep in the present study (Table 3). This may have been due to the increase in N digestibility (Table 2) as well as to a better utilization of the dietary N. Proteolytic bacteria count was increased [35] and the flow of non-microbial non-ammonia N tended to be higher for cows fed YC [27].

Regarding the effect of YC on lamb performance it was found that YC non-significantly improved ADG. Results of growth pattern agreed with those of N balance. The YC was found in other studies to improve milk yield in dairy cows' [27]. Others reported no effect with dairy cows' [26] or dairy ewes and goats [17]. Feeding YC increase the mean group feed intake; differences, however, were not tested statistically due to the group feeding system applied in the present study. Others [32, 34] reported similar results, while [17] reported no effect of YC on DM intake. Therefore, it could be recommended that YC be used in feeding the growing lambs at the studied levels in order to have better growth performance. However, more studies with higher YC levels as well as with different animal breeds are still necessary for greater clarification.

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تأثير إضافة الخميرة إلى أعلاف الغنم في الأداء الإنتاجي وكفاءة الهضم والامتصاص النيتروجيني وتخمرات الكرش

بركات محمد أحمد و محمود سيد أحمد صلاح

قسم إنتاج وتربية الحيوان، كلية الزراعة والطب البيطري، جامعة الملك سعود

فرع التقسيم بريدة - ص.ب. ١٤٨٢ - المملكة العربية السعودية

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ملخص البحث. أجريت تجربتان لدراسة تأثير إضافة مستويات مختلفة من الخميرة إلى أعلاف الغنم، تم في التجربة الأولى قياس معاملات الهضم ومعايير تخمرات الكرش والامتصاص النيتروجيني باستخدام ثلاثة كباش مفستلة موزعة في نظام المربع اللاتيني 3×3 . غذيت الحيوانات فردياً في صناديق الهضم على عليقة أساسية مكونة من الشعير والنخالة ودريس حشيشة الرودس مضافاً إليها الخميرة بتركيزات صفر، ٤، ٨ جم خميرة للرأس الواحدة يومياً. تكونت كل فترة تجريبية من فترتين؛ الأولى مدة أسبوعين كفترة تمهيدية بينما الثانية كفترة تجريبية لمدة أسبوع تم فيها جمع عينات الروث والبول كميًا. تم أيضاً جمع عينات سائل الكرش خلال الأيام الثلاثة الأخيرة من الفترة التجريبية قبل الأكل مباشرة ثم بعد ١، ٣، ٦ ساعات بعد الأكل لدراسة معايير تخمرات الكرش. أما التجربة الثانية فقد تم استخدام عدد أربعة وعشرين حملاً نامياً بمتوسط وزن ١٤ كجم قسمت عشوائياً إلى ثلاث مجموعات، بكل منها ثمانية حملاً، غذيت في مجموعات على نفس العلائق التجريبية المستخدمة في التجربة الأولى. قيس أوزان الحيوانات كل أسبوعين، بينما تم تقدير كميات الغذاء المستهلك أسبوعياً، ثم تم حساب معدلات النمو والكفاءة الغذائية.

أشارت النتائج إلى تحسن معاملات الهضم للمادة الجافة والبروتين الخام والألياف الخام تحسناً معنوياً نتيجة إضافة الخميرة إلى العلائق التجريبية مما أدى إلى ارتفاع القيمة الغذائية للأعلاف مقدرة في صورة مواد مهضومة كلية، و بروتين مهضوم أو محتوى الطاقة الممتلئة. أدت التغذية على الخميرة إلى تحسن الامتصاص

النيتروجيني وتخمرات الكرش خاصة إنتاج الأحماض الدهنية الطيارة التي تدل على ارتفاع نشاط الكائنات الحية الدقيقة بالكرش. كما تحسنت معدلات النمو وزادت كميات الغذاء المستهلك في المجموعات التجريبية المغذاة على العلائق المضاف إليها الخميرة، ولكن هذه التحسنات لم ترق إلى مستوى المعنوية في التحليل الإحصائي.