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EFFECT OF DRY YEAST (*Saccharomyces cerevisiae*) IN SHEEP RATIONS DIFFERING IN THEIR ROUGHAGE TO CONCENTRATE RATIO ON DIGESTION, NITROGEN BALANCE AND RUMEN FERMENTATION.

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SUMMARY

The effect of adding dry yeast (*Saccharomyces cerevisiae*, YC) to sheep rations was investigated in two trials. The 1st was an *in vitro* study to test the effect of YC at different levels (0, 2, 4, 6, 8 and 10g dry yeast/kg) on the disappearance of feed dry- and organic-matter (*IVDMD* and *IVOMD*). Rumen liquor used in this trial was obtained from four fistulated rams fed good quality hay of Rhodes grass. The 2nd trial was to determine the nutrient digestibility, N-balance and feeding value of the experimental rations as well as rumen fermentation *in vivo* (in terms of pH, VFA and NH_3-N concentrations). Four fistulated rams were distributed in a Latin square design and fed 4 different rations. Two rations differed in their roughage to concentrate ratio (70:30 and 30:70) were used in the experimental groups i.e.. high roughage (HRO) and high concentrate (HCO). respectively. Both rations were either used without or with (HRW and HCW) yeast culture supplementation at the level of 10g/head/d. Results of experiment 1 revealed that the addition of yeast culture at all levels used significantly improved the *IVDMD* and *IVOMD*. The highest values were reported with the level of 10g/d. *In vivo* digestibility of DM, CP and CF was also increased due to the addition of YC, which lead to an increase in the nutritive value (TDN and DCP). Nitrogen balance as well as the rumen microbial activity was also improved with the addition of YC. Sheep fed HCW ration showed higher VFA concentration followed by those fed the HCO. while those in-groups HRO and HRW were the least. The effect was more pronounced with the high roughage ration compared with the high concentrate ration.

Keywords: *digestibility, N-balance, rumen fermentation, dried yeast, sheep.*

INTRODUCTION

The use of yeast cultures (YC) as a feed additive has been increasing during the last decade. These probiotics are live microbial feed supplements, which beneficially affect the host animal by improving its intestinal microbial balance (Nunes, 1994). Probiotics have been used as growth promoters to replace the widely used antibiotic and synthetic chemical feed supplements (Higginbotham and Bath, 1993; Brydt *et al.*, 1995; Sumeghy, 1995; Strzetelski, 1996). *Lactobacilli and streptococci* are the most commonly used genera of microorganisms in the production of probiotics (Sandine, 1979 and Saucier *et al.*, 1992). *Saccharomyces cerevisiae* (as live yeast culture) was reported to balance the energy and the acid-base metabolism in dairy cattle, resulting in a significantly higher milk production (Brydt *et al.*, 1995).

The most reproducible effect of dietary yeast supplementation is the increase in bacterial numbers in the rumen, which is central to the action of the yeast in improving ruminant

productivity (Wallace and Newbold, 1992). Yeast has been shown to provide nutrients that stimulate the growth of certain rumen microorganisms such as the lactic acid-utilizing rumen bacterium *Selenomonas ruminantium* (Nisbet and Martin, 1990 and 1991). Newbold *et al.*, (1996) reported that *S. cerevisiae* stimulation of rumen bacteria depends on its respiratory activity, which allow it to scavenge O_2, thus protecting the strictly anaerobic bacteria (Rose, 1987). Its content of malic acid (Nisbet and Martin, 1990 and 1991) has little to do with its action. Yeast also provides vitamins to support the growth of rumen fungi (Chaucheyras *et al.*, 1995). The presence of respiring yeast, therefore, would be predicted to be beneficial to the rumen microflora. The present work was carried out to study the effect of adding YC to sheep rations differing in their roughage to concentrate ratio on their nutrient digestibility *in vitro* and *in vivo*. N-balance and rumen fermentation parameters (pH, VFA and ammonia-N) were also determined in order to explain the positive effect of the yeast culture.

MATERIALS AND METHODS

Two experiments were carried out in order to determine the effect of yeast-culture supplementation on *in vitro* and *in vivo* digestion kinetics in sheep.

Experiment 1: In vitro DM and OM disappearance:

A series of laboratory treatments was made to study their effects on *in vitro* DM and OM disappearance. Treatments were dry yeast (*Saccharomyces cerevisiae*, YC) supplementation at the levels of 0, 2, 4, 6, 8 and 10g dry yeast/kg of the basal ration in order to determine the best addition level. The basal ration contained Rhodes grass hay, wheat bran and barley grains at the ratio of 2:1:1, respectively (Table 1). The basal ration was dried and ground to pass through a 1mm screen. *In vitro* DM and OM digestibility were determined using the two stage technique of Tilly and Terry (1963) as modified by Ahmed (1989). Rumen liquor was obtained from four fistulated Naemy (local Saudi Arabian breed) rams (40 kg ABW) maintained on a basal ration of Rhodes grass hay (good quality) for at least 21 days. Rumen fluid was withdrawn from the animals 4-hr after feeding and strained through four layers of cheesecloth. The determination was repeated four times with each run.

Experiment 2: In vivo digestibility, N-balance and rumen fermentation

The above mentioned fistulated adult rams were used in a digestibility trial to determine the nutrient digestibility, N-balance and feeding value of the experimental rations as well as the rumen fermentation in a 4×4 Latin square design and fed 4 different rations. Two rations differing in their roughage to concentrate ratio (70:30 and 30:70) were used in the experimental groups i.e., high roughage (HRO) and high concentrates (HCO), respectively. Both rations were either used without or with (HRW and HCW) yeast culture supplementation at the level of 10g/head/d. Animals were fed separately in individual cages. The chemical composition of the ingredients and experimental rations is presented in Table (1). Animals' requirements were met according to Church (1977). Dry yeast culture (*Yea-Sacc. Saccharomyces cerevisiae, product of Alltech Biotechnology Center, Kentucky, USA*) was included in the ration by simple mixing with the concentrate part of the rations just before feeding. Animals were kept and fed in individual metabolic cages allowing a separate collection of feces and urine as described by Maynard *et al.* (1979). Animals were fed twice daily and water was available at all times. Animals were adapted to the cages for two weeks as a preliminary period followed by a collection period of one week. During the

Table 1. The proximate analysis of the feed ingredients used in formulation of the basal ration used in the present study.

Item	Barley	Wheat bran	Rhodes hay	Basal ration[1] Expt. 1	HRO[2] Expt. 2	HCO[3] Expt. 2
Dry matter, DM	93.20 (100)	90.41 (100)	91.10 (100)	91.45 (100)	91.30 (100)	91.59 (100)
Organic matter, OM	90.35 (96.94)	84.89 (93.89)	77.90 (85.51)	82.76 (90.50)	80.81 (88.51)	84.71 (92.49)
Crude protein, CP	10.66 (11.44)	11.79 (13.04)	7.35 (8.07)	9.29 (10.16)	8.51 (9.32)	10.06 (10.98)
Ether extract, EE	2.06 (2.21)	2.71 (3.00)	1.12 (1.23)	1.75 (1.91)	1.50 (1.64)	2.01 (2.19)
N-free extract, NFE	73.41 (78.76)	59.58 (65.90)	40.28 (44.22)	53.39 (58.38)	48.14 (52.73)	58.63 (64.02)
Crude fiber, CF	4.22 (4.53)	10.82 (11.97)	29.15 (32.00)	18.34 (20.05)	22.66 (24.82)	14.01 (15.29)
Ash	2.85 (3.06)	5.51 (6.09)	13.20 (14.48)	8.69 (9.50)	10.49 (11.49)	6.88 (7.52)

Values between parenthesis are on DM basis.

[1]Basal ration used in the experiment 1, containing 50% Rhodes hay, 25% wheat bran and 25% barley grain.

[2]HRO, high roughage ration used in experiment 2; containing 70% Rhodes hay, 15% wheat bran and 15% barley grain.

[3]HCO, high concentrate ration used in experiment 2; containing 30% Rhodes hay, 35% wheat bran and 35% barley grain.

collection week feces and urine were collected and were sampled for analysis. Chemical analyses were conducted according to A.O.A.C. (1980).

For the rumen fermentation study, animals were fistulated prior to the experiment. Rumen samples were collected during the three days following the collection period of the digestibility trial at 0, 1, 3 and 6 h post-morning feeding to determine the production of volatile fatty acids (VFA) and ammonia-nitrogen (NH3-N) concentration. Rumen fluid 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 H_2SO_4 (50%v/v) to retard ammonia loss. Samples were frozen for subsequent analysis for ammonia according to Al-Rabbat *et al.*(1971), and total volatile fatty acids as described by Warner (1964).

Statistical analysis:

Data were analyzed by ANOVA for Latin square design using GLM procedure of the SAS program (1996) 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} dietary treatment and e_{ijk} is the residual error assumed to be normally and independently distributed.

Duncan's test (1955) was used to compare the treatment means.

RESULTS AND DISCUSSION

Results of *Experiment 1* (Table 2) revealed that the *in vitro* disappearance of DM and OM were significantly ($P<0.05$) increased with YC supplementation at all levels used. The increases in *IVDMD* and *IVOMD* were linearly correlated with the increase in the YC level. The correlation coefficient (R^2) was 0.98 for *IVDMD* and 0.99 for *IVOMD*. *IVDMD* increased by 1.09 unit for each unit of supplementation while it was only 0.94 unit for *IVOMD* (Table 2).

Table 2. The *in vitro* dry matter (*IVDMD*) and organic matter (*IVOMD*) disappearance of the basal ration as affected by the yeast culture supplementation level.

Yeast culture level	*IVDMD*	*IVOMD*
0 g	65.76 ± 0.74^{a}	67.56 ± 0.87^{a}
2 g	67.35 ± 0.85^{ab}	68.90 ± 0.78^{a}
4 g	69.87 ± 0.87^{b}	71.21 ± 0.91^{ab}
6 g	72.01 ± 0.86^{bc}	73.45 ± 0.86^{b}
8 g	74.56 ± 0.97^{c}	74.98 ± 0.75^{bc}
10g	75.78 ± 0.92^{c}	76.58 ± 0.98^{c}
R^2	0.98	0.99
	$y = 65.76 + 1.09\ x$	$y = 67.56 + 0.94\ x$

[a,b,c] Values having different superscript within each column are significantly different ($P<0.05$)

The *in vivo* digestibility (*Experiment 2*) of DM was significantly ($P<0.05$) improved by the supplementation of YC only with the high roughage diet (Table 3) while it was not affected by YC with the high concentrate diet. Digestibility of CP followed the same trend; however, the effect of YC failed to be significant. The

digestion coefficient of CF was also improved by the YC supplementation and the effect was significant ($P<0.05$) and was more pronounced with the high roughage ration. In general, CF digestibility was higher with the high roughage than with the high concentrate, with or without YC. Supplementation of YC had no effect on the digestibility of NFE and EE; the digestibility of NFE was non-significantly lower with the high roughage than with the high concentrate ration. Yeast culture has been observed to improve the digestibility of most nutrients (Wiedmeier *et al.*, 1987; Williams *et al*, 1991; Wohlt *et al.*, 1991; Harris *et al.*, 1992; Dawson, 1993; Robinson, 1997; Putnam *et al*, 1997). Robinson (1997) reported that supplementation of yeast culture in the diet increased net digestion in the fore-stomach, particularly of fiber leading to increased energy output. Yoon and Stern (1996) 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 (Wiedmeier *et al.*, 1987 and Newbold *et al.*, 1997) and/or activity (Erasmus *et al.*, 1992 and Dawson, 1993) of rumen cellulolytic bacteria. Proteolytic bacteria counts were also stimulated by yeast culture (Yoon and Stern, 1996).

The increase in digestibility lead to an increase in the feeding values (Table 3). Total digestible nutrients (TDN) increased from 62.76% in the high roughage ration to 64.87% and from 68.74% in high concentrate ration to 69.84% due to the YC supplementation. However, the effect of YC was significant only with HR ration. The HC rations (with or without YC) had more TDN than the HR ration. The same trend was found for digestible crude protein (DCP) being 6.28 and 7.20% for HR rations and 7.99 and 8.06% for HC rations without and with YC supplementation.

Table 3. The *in vivo* digestion coefficients and nutritive value of the experimental diets as affected by the addition of yeast culture.

Item	Experimental rations[1]			
	HRO	HRW	HCO	HCW
Digestion coefficients				
DM	63.3±1.21^{a}	66.9 ± 0.85^{b}	69.2 ± 1.02^{c}	70.7 ± 1.07^{c}
CP	67.4±1.13^{a}	69.3 ± 0.87^{a}	72.8 ± 0.96^{bc}	73.4 ± 1.19^{c}
EE	64.6±1.35^{a}	64.8 ± 1.28^{a}	65.6 ± 1.97^{a}	65.9 ± 1.26^{a}
NFE	74.5±1.05^{a}	75.4 ± 1.31^{a}	77.3 ± 1.63^{a}	78.1 ± 1.47^{a}
CF	59.7±1.03^{b}	65.5 ± 0.76^{c}	52.5 ± 0.98^{a}	55.8 ± 0.94^{b}
Nutritive value (on DM basis)[2]				
TDN	62.76±1.41^{a}	64.87 ± 1.42^{b}	68.74 ± 1.34^{c}	69.84 ± 1.53^{c}
DCP	6.28±0.10^{a}	7.20 ± 0.09^{b}	7.99 ± 0.10^{ab}	8.06 ± 0.10^{b}

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

[1] HRO, high roughage ration without YC supplementation; HRW, high roughage ration with YC supplementation; HCO, high concentrate ration without YC supplementation; HCW, high concentrate ration with YC supplementation.

[2] TDN, total digestible nutrients; DCP, digestible crude protein.

To clarify the mode of action of yeast culture, ruminal microbial activity was evaluated as pH and concentrations of ammonia-N and volatile fatty acid (VFA). The pH values of rumen liquor are illustrated in Fig (1). No differences in

the pH values were found due to the YC supplementation. Yoon and Stern (1996) and Putnam *et al.* (1997) have reported similar results. The high concentrate rations caused a significantly ($P<0.05$) sharper drop in pH than the high roughage rations especially at 3hr-post feeding. At 6hr post-feeding the pH values were nearly similar in all the experimental rations.

Rumen ammonia-N concentration of sheep fed different diets is illustrated in Fig. (2). Before feeding (at zero time) ammonia-N was lower with the HR ration (with and without YC) than the HC rations at all times studied. The YC supplementation lead to a non-significant increase in ammonia-N concentrations with both rations (HR and HC) at all times. After the first hour, ammonia-N concentrations started to decline linearly to reach the minimum at 6-h post-feeding. Putnam *et al.* (1997) and Ahmed and Salah (2000) reported similar trends.

Concentrations of VFA as affected by the experimental diets are shown in Fig. (3). The lowest level was reported before feeding for all dietary treatments. At 1h after feeding, sheep fed HCW ration showed higher VFA concentration followed by those fed the HCO, while those in groups HRO and HRW were the least. The same trend was observed at 3h then declined at 6h. The increase in VFA concentrations at 3h post-feeding lead to the decreases observed in pH values (Fig. 1). Ahmed and Salah (2000) concluded similar effect of YC at two levels (4 and 8g/head/d). The effect of YC supplementation was positive and more pronounced with the HR diets.

In general the microbial activity was increased with the advance in time reaching the maximum activity at 3h after feeding then declined (Figures 1-3). Similar trend was reported by (Taie *et al.*, 1998). This may have been due to the increase in the bacterial counts and activity (Erasmus *et al.*, 1992; Yoon and Stern, 1996; and Putnam *et al.*, 1997) and the stability of the ruminal environment (Lyons, 1994). The differences between our results and other published data (Hadjipanayiotou *et al.*, 1997) could be attributed to differences in the quantities used and/or different strains of YC. Newbold *et al.* (1996) reported that some strains of yeast are effective whereas others are not. They suggested that 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 O_2 from rumen fluid. The amount of O_2 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 (Czerkawski *et al.*, 1969 and Newbold *et al.*, 1996). Oxygen is known to be toxic to anaerobic bacteria and it inhibits the growth of rumen bacteria in pure culture studies (Loesche, 1969 and Marounek and Wallace, 1984) and the adhesion of cellulolytic rumen bacteria to cellulose (Roger *et al.*, 1990). The presence of respiring yeast, therefore, would be predicted to be beneficial to the rumen microorganisms.

Results of the nitrogen balance are presented in Table (4). Sheep fed HC had significantly ($P<0.05$) more nitrogen intake (NI) than those fed the HR diets. That was due to the higher protein contents of the HC diet (Table 1). Difference was significant ($P<0.05$). Fecal N did not significantly differed in all the experimental groups. The higher intake with the same fecal output lead to more digested N in-groups of HC. Sheep fed the HC rations secrete more N in the urine than those fed the HR (either with or without YC). N balance was the lowest with sheep fed the HRO followed by

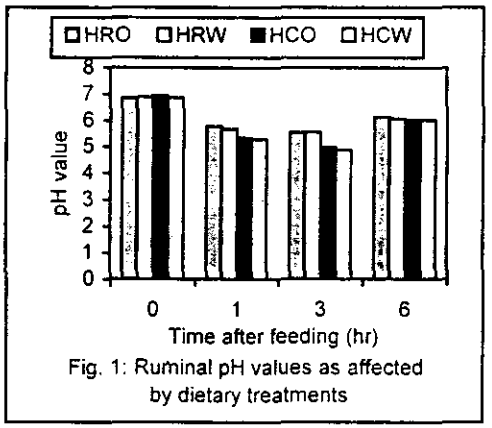

Fig. 1: Ruminal pH values as affected by dietary treatments

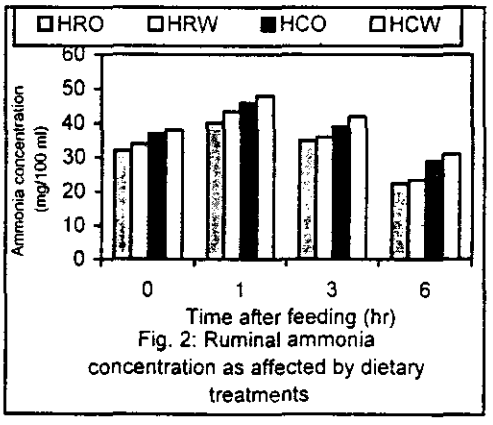

Fig. 2: Ruminal ammonia concentration as affected by dietary treatments

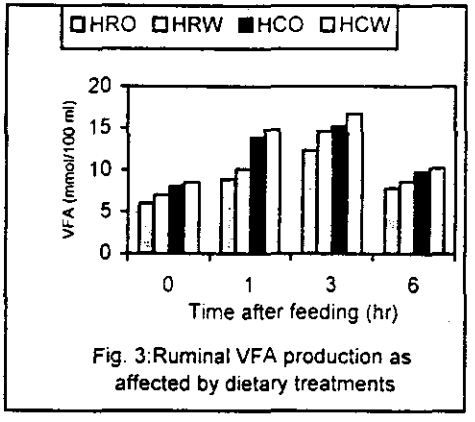

Fig. 3:Ruminal VFA production as affected by dietary treatments

Table 4. Nitrogen balance (g/head/day) of sheep as affected by dietary yeast culture

Item	Experimental rations[1]			
	HRO	HRW	HCO	HCW
N intake (NI)	19.06 ± 1.11^{a}	20.02 ± 1.54^{a}	22.53 ± 1.76^{b}	23.49 ± 1.07^{b}
Fecal N (FN)	6.21 ± 0.14^{a}	6.15 ± 0.18^{a}	6.13 ± 0.24^{a}	6.25 ± 0.19^{a}
Urinary N (UN)	7.73 ± 0.10^{a}	7.78 ± 0.12^{a}	8.97 ± 0.08^{b}	9.20 ± 0.11^{b}
N balance (NB)	5.12 ± 0.04^{a}	6.09 ± 0.06^{b}	7.43 ± 0.04^{c}	8.04 ± 0.06^{c}
N digested (ND)	12.85 ± 0.16^{a}	13.87 ± 0.16^{a}	16.40 ± 0.23^{b}	17.24 ± 0.25^{b}
NB/NI, %	26.86 ± 0.22^{a}	30.42 ± 0.16^{b}	32.98 ± 0.11^{c}	34.23 ± 0.21^{c}
NB/ND, %	39.84 ± 0.31^{a}	43.91 ± 0.29^{b}	45.30 ± 0.34^{bc}	46.64 ± 0.36^{c}

[a,b,c] Values having different superscripts within each row are significantly different ($P<0.05$)
[1]HRO, high roughage ration without YC supplementation; HRW, high roughage ration with YC supplementation; HCO, high concentrate ration without YC supplementation; HCW, high concentrate ration with YC supplementation.

those on HRW and the highest was that of HCW which was non-significantly higher that the HCO. Nitrogen balance as a percentage of NI was better with HC groups (32.98 and 34.23%) followed by HRW (30.42) and the least was the HRO group (26.86); differences were significant. When NB was calculated as percentages of N-digested (ND), the same pattern was obtained. This means that the YC had more pronounced effect with HR than with HC diets. This may have been due to the increase in N digestibility (Table 2) as well as to a better utilization of the dietary N. Similar effect of YC was reported by Ahmed and Salah (2000) who found that YC supplementation at the level of 8g/head/day tended to improve the N balance.

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تأثير إضافة الخميرة الجافة إلى علائق الأغنام المحتوية على نسب مختلفة من الأعلاف الخشنة إلى المركزة على الهضم والاتزان النيتروجيني وتخمرات الكرش.

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أجريت تجربتان بهدف دراسة تأثير إضافة الخميرة إلى علائق الأغنام، تم في الأولى قياس تأثير إضافة تركيزات متتالية من الخميرة (من صفر وحتى ١٠جم /كجم عليقة) على معاملات اختفاء المادة الجافة والعضوية معمليا حيث تم الحصول على سائل الكرش من أربعة كباش مزودة بفستيولا في الكرش ومغذاة على دريس جيد من حشيشة الرودس. في التجربة الثانية تم قياس الهضم والاتزان النيتروجيني وتخمرات الكرش (الحموضة وتركيز الأمونيا والأحماض الدهنية الطيارة) باستخدام الكباش المذكورة موزعة في نظام تجريبي المربع اللاتيني ٤ × ٤ غذيت فرديا في صناديق الهضم على أربعة علائق مختلفة النسبة بين العلف المركز والمالئ (٧٠:٣٠ ، ٧٠:٣٠) مكونة من الشعير والنخالة ودريس حشيشة الرودس إما بدون إضافة أو مضافا إليها الخميرة بتركيز ١٠ جم خميرة للرأس الواحدة يوميا. تكونت كل فترة تجريبية من فترتين الأولى مدة أسبوعين كفترة تمهيدية بينما الثانية كفترة تجريبية لمدة أسبوع تم فيها جمع عينات الروث والبول كميا. تم جمع عينات سائل الكرش خلال الأيام الثلاثة الأخيرة من الفترة التجريبية قبل الأكل مباشرة ثم عند ١ ، ٣ ، ٦ ساعات بعد الأكل لدراسة تخمرات الكرش.

أشارت نتائج التجربة الأولى إلى تحسن معاملات اختفاء المادة الجافة والمادة العضوية معمليا بارتفاع تركيز الخميرة حتى وصلت إلى أقصى تأثير معنوي عند تركيز ١٠جم خميرة/كجم من العليقة . كما تحسنت معاملات هضم المادة الجافة والبروتين الخام والألياف الخام في التجربة الثانية تحسنا معنويا نتيجة إضافة الخميرة مما أدى إلى ارتفاع القيمة الغذائية للعلائق مقدرة في صورة مجموع مواد مهضومة كلية أو بروتين مهضوم. كما أدت التغذية على الخميرة إلى تحسن الاتزان النيتروجيني وتخمرات الكرش خاصة تركيز الأحماض الدهنية الطيارة التي تدل على ارتفاع نشاط الكائنات الحية الدقيقة بالكرش خاصة عند إضافتها إلى العلائق عالية المحتوى من الأعلاف المالئة عن تلك المرتفعة المحتوى من المركزات.