Effect of Feeding *Saccharomyces cerevisiae* and/or *Aspergillus oryzae* on Nutrient Utilization and Rumen Fermentation Characteristics of Sheep

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ABSTRACT

This experiment was conducted to evaluate the potential impacts of commercial *Saccharomyces cerevisiae* SC (Probio-Sacc®) and/or *Aspergillus oryzae* (AO) extract (Amaferm®) supplementation on dry matter intake (DMI), digestion coefficients of nutrients, nitrogen balance and rumen fermentation characteristics. A digestibility trial was carried out using twelve adult male Barki sheep allocated into four groups of 3 animals each. The control group was fed a basal diet without supplement, the SC diet was supplemented with 0.5g of SC/h/d, the AO diet was supplemented with 0.5 g of AO/h/d and the SC+AO diet was supplemented with a combination of 0.5g of SC and 0.5 g of AO/h/d.

The results showed that inclusion of AO and a combination of SC+AO increased (P<0.05) DMI by 5% and 9%, respectively as compared to the control group. SC supplement increased (P<0.05) apparent total tract digestion coefficients of dry matter (DM) and organic matter (OM). Supplementation of AO increased significantly (P<0.05) the apparent total tract digestion coefficient of ether extract (EE), while the combination of SC+AO decreased (P<0.05) digestibility of EE. The apparent total tract digestion coefficient of crude fiber (CF) were enhanced (P<0.05) by 15% and 26% due to SC and SC+AO supplementations, respectively as compared to the control group. Supplementation of SC, AO or their combination had no significant effects on the apparent total tract digestion coefficients of neutral detergent fiber (NDF), while those of acid detergent fiber (ADF) were improved (P<0.05) as compared to the control group. However all treatments had no significant impacts on digestion coefficients of crude protein (CP), total digestible nutrients (TDN) and digestible crude protein (DCP) as compared to the control group. Although the combination of SC+AO supplementation improved (P<0.05) nitrogen intake, no significant influences on fecal or urinary N execration or N balance were observed. Rumen pH and concentration of ammonia nitrogen were not affected while VFA concentration increased (P<0.05) by 24% with AO supplementation as compared to the control group. It can be concluded that combination of SC and AO has the potential to improve cell walls digestion and nutrients utilization in sheep.

Key words: *Saccharomyces cerevisiae*, *Aspergillus oryzae,* fermentation, nutrients digestibility, nitrogen balance

INTRODUCTION

Microbial products based on live yeasts, yeast cultures or *Aspergillus* species are claimed to improve ruminant performance through modulating rumen function and the activities of its microflora. Higher activity of rumen microbes induces better digestibility of dietary fiber, leading to enhancing the DMI and consequently animal performance.

Effects of such products on production traits have been documented in most domestic ruminants. The favorable influence of fungal culture on ruminal fermentation and microbial population via proteolytic, cellulolytic bacteria counts (Yoon and Sterns, 1996) and lactic acid fermenting bacteria (Beharka and Nagaraja, 1998) have been demonstrated. However, the research on fungal additives in dairy cattle has mainly focused on milk production, with contradictory results (Wallace and Newbold, 1995; Lesmeister et al., 2004). Similarly the results with SC have been variable and inconsistent probably due to feed composition, type of microbial feed additives, sources of ingredients and environmental conditions (Robinson, 2002 and Newbold, 1990).

There are few studies focused on the impacts of yeast and *Aspergillus* together on the rumen fermentation patterns and nutrient digestibility. It was hypothesized that the combination of both products may have an additive effect on stimulating rumen fermentation characteristics and plant cells digestion. Thus, the aim of this study was to evaluate effects of inclusion of AO fermentation extract and SC culture product and a combination of both on nutrients utilization and rumen fermentation characteristics in sheep.

MATERIALS AND METHODS

This study was conducted at the Milk Production Project, Animal and Fish Production Department, Faculty of Agriculture, Alexandria

University, Egypt. All analyses were carried out at the Animal Nutrition Laboratory, Department of Animal and Fish Production, Faculty of Agriculture, Alexandria University.

Animals and management:

Twelve adult male Barki sheep with an average live body weight of 41 kg±4.1 were randomly divided into four groups of 3 males each. The Control group was fed a basal diet, composed of concentrate mixture and Egyptian clover (*Alexandrium trifolum*) hay without supplement, the SC group received the basal diet plus 0.5g/h/d of Probio-Sacc® (*S.cerevisiae*, MUCL 39885 15x10^9 CFU/ g), the AO group received the basal diet plus 0.5 g/h/d of Amaferm® (*Aspergillus oryzae* fermentation extract) and the SC+AO group received the basal diet plus combination of 0.5g SC and 0.5g of AO/h/d.

The ingredients and chemical composition of the concentrate mixture and clover hay are presented in Table (1). The animals were group fed on 50: 50% roughage: concentrate at a rate of 3% of their average live body weight according to NRC (2001). The animals were housed individually in metabolic cages and had free access to fresh water. Individual intakes of clover hay and concentrate mixture were recorded daily by weighing the offered feed and refusals. During the collection period, the complete output of feces was collected in buckets and recorded. Feces samples of 100 g/kg of total weight were stored under -20˚C. All collected samples were mixed and one kilogram of the mixture was dried off at 60˚C for 72 h in a forced air oven, ground and descended through a 1-mm screen and stored at room temperature until analysis.

Urine was collected into buckets containing 100 ml of 100 ml/l (v/v) sulphuric acid to reduce pH below 3.0 and prevent bacterial destruction of urine samples. The volume of urine at each sampling was determined and sub-samples of 10 ml/100 ml of total urine were collected from individual sheep and frozen until analysis of total N.

Rumen fluid was collected via stomach tube before morning feeding on the last day of the digestibility trail and pH was measured immediately. The rumen fluid was separated from the feed particles through four layers of gauze and frozen for subsequent analyses.

2.4. Chemical analyses:

Samples were oven dried at 60°C for 24h and stored for later chemical analysis according to AOAC (1990) for dry matter (DM, method number 981.10), crude protein (CP, method number 967.03), ash (method number 942.05) and NDF and ADF fractions according to Van Soest et al.(1991) without sodium sulphite or heat-stable amylase and expressed include residual ash. Hemicellulose was calculated as the difference between NDF and ADF. Concentrations of NH_3-N and total volatile fatty acids (VFA) in rumen fluid were determined by distillation using Markham apparatus according to Preston (1995) and Warner (1964), respectively.

3.5. Statistical analysis:

Data were analyzed by the generalized linear model procedure (SAS, 2002), with the assumed model: $Y_{ij}= \mu+ F_i + e_{ij}$ where: μ is the overall mean, F_i is the treatment type, e_{ij} is the random error term. Differences among means were tested using Duncan multiple range test (Steel and Torrie, 1980).

RESULTS

Data of DMI, nutrient apparent total tract digestion coefficients and feeding values of the experimental rations with and without SC, AO or SC+AO supplements are summarized in Table (2).

Table 1: Ingredients of the concentrate mixture and chemical composition of the concentrate mixture and Egyptian clover hay on dry matter basis

Ingredients of concentrate mixture		g/kg
Ground yellow corn		400
Wheat bran		290
Cotton seed meal		200
Soy bean meal		080
Limestone		018
NaCl		010
Minerals mixture		002
Items, %	**Concentrate**	**Egyptian clover**
Organic matter	92.1	95.1
Crude protein	15.9	13.8
Ether extract	3.3	1.64
Neutral detergent fiber	48.6	64.2
Acid detergent fiber	21.8	55.8
Hemicellulose	26.8	8.3

Table 2: Means ± SE of dry matter intake (DMI), nutrients digestion coefficients and nutritive value of ration supplemented with *S. cerevisiae* (SC), *A. oryza* (AO) and their combination and fed to Braki sheep

Item	Control	SC	AO	SC+AO	P values
DMI, g/head/d	1112.8±17.6^{c}	1146.1±17.6^{bc}	1167.3±17.6^{ab}	1216.7±17.6^{a}	0.001
% Digestion coefficients of :					
Dry matter	64.3±1.4^{b}	68.6±1.4^{a}	66.3 ±1.4^{ab}	66.0±1.4^{ab}	0.019
Organic matter	64.7±1.3^{b}	69.6±1.3^{a}	67.2±1.3^{ab}	67.4±1.3^{ab}	0.047
Crude protein	69.4±1.2	69.9±1.2	68.9±1.2	68.2±1.2	0.811
Ether extract	62.3±2.4^{bc}	66.9±2.6^{b}	81.9±2.5^{a}	58.7±2.9^{c}	0.001
Crude fiber	45.9±2.3^{c}	57.9±1.9^{a}	50.7±2.0^{b}	52.8±2.0^{b}	0.001
Neutral detergent fiber	63.4±1.5	66.9±1.5	66.6±1.5	66.2±1.5	0.341
Acid detergent fiber	51.9±1.7^{b}	59.6±1.7^{a}	59.6±1.7^{a}	58.6±1.6^{a}	0.006
%Nutritive value					
TDN	62.6±1.3	66.3±1.3	64.7±1.3	64.4±1.3	0.256
DCP	10.5±0.2	10.5±0.2	10.3±0.2	10.2±0.2	0.707

Different letters (a,b) in the same row indicate significant differences (P<0.05). TDN: Total digestible nutrients; DCP: Digestible crude protein.

The combined SC and AO supplementations resulted in 9% increase (P<0.001) in DMI over the control group. Moreover, AO supplement alone had significantly (P<0.05) enhanced DMI by 5% over the control group, but SC supplement had no significant effect on DMI as compared to the control group. Apparent total tract digestibility of DM and OM increased (P<0.05) due to supplementing SC at approximately a rate of 7% above the control group. However, either AO or combined SC+AO supplement had no significant effect on apparent total tract digestibility of DM and OM. Supplementing AO increased (P<0.05) the apparent total tract digestibility of EE by 24% above that of the control group. Moreover, combined SC+AO supplement decreased (P<0.05) the apparent total tract digestibility of EE compared to either SC or AO group.

The supplementation of SC and the combined SC+AO enhanced (P<0.05) the apparent total tract digestion coefficients of CF by 15% and 26%, respectively as compared to the control group. But either supplement or their combination had no significant effect on the apparent total tract digestion coefficient of NDF, but the digestion of ADF has improved (P<0.05) by supplementing SC, AO or SC+AO as compared to the control group. However, non of the treatments had significant impacts on digestion coefficients of CP, TDN or DCP as compared to the control group.

The impacts of SC, AO or SC+AO supplementation on N intake, fecal N, urinary N and N balance are shown in Table (3). When the combined SC+AO was supplemented, N intake exhibited 7.5% increase above the control group, whereas, SC or AO supplementation had no significant effect on N intake as compared to the control group. No significant effects due to supplement of SC, AO or their combination were observed on the feces or urinary nitrogen or on N-balance as compared to the control group.

Ammonia-N, VFA concentrations and rumen pH for control, SC, AO and SC+AO supplementations are presented in Table (4). Supplementations had no significant effect on NH_3-N and rumen pH as compared to the control group. While AO supplement increased (P<0.05) VFA concentrations by 24% above that of control group.

Table 3: Means ± SE of nitrogen fractions of ration supplemented with *S. cerevisiae* (SC), *A.oryza* (AO) and their combination and fed to Braki sheep

Item	control	SC	AO	SC+AO	P values
N intake, g/d	26.8±0.59^{b}	27.5±0.59^{ba}	28.0±0.59^{ba}	29.0±0.59^{a}	0.020
Fecal N	8.2±0.63	8.3±0.63	8.7±0.63	9.3±0.63	0.647
Urinary N	8.5±1.1	8.5±1.1	9.1±1.1	8.8±1.1	0.976
Nitrogen balance, g/d	10.0±1.2	10.7±1.2	10.2±1.2	11.0±1.2	0.943

Different letters (a, b) in the same row indicate significant differences (P<0.05).

Table 4. Means ± SE of NH_3-N, volatile fatty acids (VFA) concentration and pH values of ration supplemented with *S. cerevisiae* (SC), *A.oryza* (AO) and their combination and fed to Braki sheep

Item	control	SC	AO	SC+AO	P values
NH_3-N, mg/dL	21.5±2.4^{ab}	29.1±2.4^{a}	20.8±2.7^{b}	24.1±2.4^{ab}	0.021
VFA, meq/dL	6.8±0.65^{b}	8.2±0.59^{ab}	9.0±0.59^{a}	8.3±0.59^{ab}	0.048
Rumen pH values	7.0±0.06	7.0±0.06	6.9±0.06	6.9±0.06	0.786

DISCUSSION

Microbial additives such as yeasts or AO are widely used in ruminant nutrition to manipulate rumen fermentation and improve animal performance (Wallace, 1994). Yeast culture products and AO fungal fermentation extracts are widely used to improve the performance of livestock due to their act of modifying ruminal fermentation and stimulation of ruminal bacterial, protozoal and fungal growth (Williams et al., 1991; Erasmus et al., 1992). Higher activity of rumen microbes will enhance the digestibility of dietary fiber and lead to higher DMI. Feed intake is, therefore, regareded as being fungal feed additive driven. Numerous factors are known to influence appetite, but those related to SC and AO supplementations in ruminants rations are palatability, rate of fibre digestion, rate of digesta flow and protein status (Harris and Lobo, 1988; Yoon and Stern, 1996). The enhanced DMI, which may drive production responses to microbial feed additives is most likely due to an improved rate of breakdown of feedstuffs in the rumen. Addition of yeasts to ruminant diets increased DMI and consequently yield of milk and milk components between 2 and 5%, but slightly decreased feed efficiency (Robinson and Erasmus, 2009).

The stimulatory effects of SC products to nutritive efficiency were exhibited as area availability for energy production rather than for protein formation. Improved fiber digestibility and production seem to be one of advantages of supplementing yeast products which improve metabolic energy supply and explain the higher DMI (Jouany, 2006).Kholif et al., (2000) and Moallem et al., (2009) reported an improve in fiber digestion when added yeast products. The inclusion of yeast products to the diet benefits fiber digestion in ruminants through stimulating cellulolytic bacteria and preventing the decline in rumen pH caused by decreasing lactic acid production or increasing the bacterial utilization of lactic acid or both (Bertin and Andrieu 2005 and Robinson and Erasmus, 2009).

The enhanced DM and OM digestibility driven by the improved fermentation activities of the rumen bacteria, especially cellulolytic strains appeared to increase by supplementing SC (Wiedmeier et al., 1987), which is capable to scavenge excess oxygen (Jouany, 2001) to create optimal environment for rumen anaerobic bacterial activities. Nevertheless, some of *S. cerevisiae* strains lack the ability to have stimulatory effects on rumen fermentation (Zinn and Borquez, 1993 and Yoon and Stern, 1996).

The increased rates of ruminal fiber digestion consequently lead to improve in ruminant productivity. Similarly Yang et al., (2004) reported that supplementing SC increased cell wall fibre degradation by the stimulated cellulolytic bacteria.

The increase of apparent digestibility of ADF associated with supplementing SC, AO or SC+AO is a result of increments in total and cellulolytic bacterial populations observed with the addition of yeast culture (Dawson et al., 1990) or fungal culture (Fondevilla et al., 1990), whereas NDF did not respond significantly to supplementations. Roa et al. (1997) indicated that the quality of the forage may alter the effects of yeast culture on NDF digestion. However, Firkins et al. (1990) observed no differences in ruminal OM and NDF digestion when AO was supplemented, while Gomez-Alarcon et al. (1990) reported increases in ruminal DM and NDF digestion in ruminally and duodenally cannulated cattle when AO was supplemented, though results were inconsistent across trials.

Combining SC and AO exhibited significant increase in nitrogen intake concurrent with DMI but without significant effects on N excreted in feces, urine or retained N. In contrary, El-Ashry et al., (2003); Ahmed and Salah (2006); Ismaiel et al., (2010) and Sallam et al., (2014) reported that microbial feed additives brought about less excretion of urinary and fecal nitrogen, which led to improvement in nitrogen balance.

Little is known about how SC and AO work. Multiplication of bacterial numbers by SC supplementation may increase the rate of substrate fermentation and microbial protein synthesis (Ryan and Gray, 1989). In theory, this should enhance ammonia uptake fermentations (Wallace and Newbold, 1995) but results in that aspect have been inconsistent. Hadjipanayiotou et al., (1997) claimed that the use of *S. cerevisiae* had no effect on nutrient digestibility, whereas Plata et al., (1994) found positive *in vivo* or *in situ* responses. Moreover, Hobson and Stewart (1997) concluded that research on microbial feed additives is often frustrating because responses are always small and highly variable.

Results of the lacked effect of microbial additives on NH_3-N or *in situ* protein degradability are in agreement with those of Erasmus et al., (1992); Sievert and Shaver, (1993). Also, Firkins et al., (1990) reported increases in acetate when AO was used in agreement with the current results that VFA concentration recorded 24% increase above the control group. Reduced rumen outflow rates may explain the increase in concentration in ruminal VFA, although Wiedmeier et al. (1987) showed that neither yeast nor fungal cultures used alone or in combination affected the liquid outflow or particulate rates of passage in non-pregnant and non-lactating Holstein cows.

CONCLUSION

Supplementing sheep rations with a combination of SC + AO fermentation extract improved DMI, nitrogen intake, cell walls digestibility compared to supplementing either of them. Stimulation of ruminal fermentation can be beneficial in terms of providing more energy for microbial multiplication and consequently maintenance and production activities of the host animal. However, the host response to addition of such supplements have been highly variable and is influenced by the composition of the diet which much remains to be elucidated regarding the dose and the diet composition, which may affect lactic acid concentration and rumen pH.

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الملخص العربى

تأثير إضافة الخميرة وفطر الأسبيراجلس علي الإستفادة من المكونات الغذائية وخصائص تخمرات الكرش في الأغنام

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أجريت هذه التجربة لتقييم تأثير إضافة بعض الإضافات الميكروبية التجارية مثل الخميرة([Probio-Sacc] و/ أو مستخلص فطرالأإسبيراجلس [Amaferm] على المادة الجافة المأكولة، معاملات هضم العناصر الغذائيـة، الإتـزان الازوتى وخصائص تخمرات الكرش.

أجريت تجربة الهضم بإستخدام ١٢ رأس من أغنام البرقى قسمت إلى أربع مجـاميع (ثـلاث حيوانـات لكـل مجموعة) كالأتي:

١- المجموعة الأولى (القياسيه) غذيت على العليقة الأساسية بدون إستخدام أى إضافات.
٢- المجموعة الثانية (مجموعة الخميرة) غذيت على العليقة الأساسية + ٠.٥ جم من خميرة (SC)/ رأس/ يوم.
٣- المجموعة الثالثة (مجموعة فطر الإسبيراجلس)غذيت على العليقة الأساسية + ٠.٥ جم من مسـتخلص (AO)/ رأس/ يوم.
٤- المجموعة الرابعة (مخلوط من الخميرة وفطر الإسبيراجلس) غذيت على العليقة الأساسـية + (٠.٥ جـم مـن الخميرة + ٠.٥ جم من فطر الإسبيراجلس/ رأس/ يوم).

أوضحت النتائج أن إستخدام مخلوط من الخميرة وفطر الإسبيراجلس وفطر الإسبيراجلس بمفردة أدت إلى زيادة معنوية فى كمية المادة الجافة المأكولة يومياً بنسبة ٩% و٥% على الترتيب مقارنة بالمجموعـة القياسـيه. إضـافة الخميرة أدى إلى زيادة معنوية فى معامل هضم المادة الجافة والمادة العضوية بينما إضافة فطر الإسـبيراجلس أدت إلى زيادة معنوية فى معاملات هضم المستخلص الإيثيرى فى حين انها انخفضت معنويا بإضافة مخلـوط الخميـرة وفطر الإسبيراجلس. حدث تحسن معنوى لمعامل هضم الألياف الخام بنسبة ١٥% و٢٦% نتيجة إضـافة مخلـوط الخميرة وفطر الإسبيراجلس والخميرة بمفردها على الترتيب بالمقارنة بالمجموعة القياسيه. حدثت زيادة معنوية فـى معامل هضم الألياف القابلة للذوبان فى الجوهر الحامضى بإضافة كلاً من الخميرة أو الفطـر أو مخلـوط الخميـرة والفطر مقارنةَ بالمجموعة القياسيه ومع ذلك لم يظهر أى تأثير معنوى للإضافات على معامل هضم الألياف القابلـة للذوبان فى الجوهر المتعادل، البروتين الخام، مجموع العناصر الغذائية المهضومة والبـروتين المهضـوم مقارنـة بالمجموعة القياسيه. بالرغم من أن إضافة مخلوط الخميرة وفطر الإسبيراجلس حسنت كميه النيتـروجين المـأكول معنوياً فإن جميع المعاملات لم يكن لها تأثير معنوى على النيتروجين المفرز فى الروث أو البول او علـى الإتـزان الأزوتى. جميع المعاملات لم يكن لها تأثير على تركيز الأمونيا والأس الهيدروجيني (pH) في الكرش بينما حـدثت زيادة معنوية في تركيز الأحماض الدهنية الطيارة بنسبة ٢٤% بإستخدام فطر الإسـبيراجلس مقارنـة بالمجموعـة القياسيه.

يستنتج من هذه الدراسة أن إضافة كل من الخميرة وفطر الإسبيراجلس ساعدت على تحسـين هضـم الجـدر الخلوية والإستفادة من العناصر الغذائية فى المجترات.