Dept. of Nutrition and Clinical Nutrition,
Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt.
E-mail: abdelrheem@yahoo.com

EFFECTS OF AUTOCLAVED YEAST CULTURES *(SACCHAROMYCES CEREVISIAE)* AND LIVE YEAST ON RUMINAL FERMENTATION *IN VITRO*

(With 7 Tables and One Figure)

By

SH.M. ABDEL-RAHEEM, WAGEHA A. AWAD*, C. IBEN** and J. BÖHM**

*Dept. of Animal Hygiene, South Valley University, Qena, Egypt.

**Institute of Animal Nutrition, Dept. of Farm Animals and Veterinary Public Health, University of Veterinary Medicine, Vienna, Austria

(Received at 26/11/2011)

أثر إضافة كل من مستنبت الخميرة المعقمة والخميرة الحية علي مقاييس التخمر في الكرش معمليا

شريف محمد عبد الرحيم ، وجيهة عوض ، كرستينا إيبن ، يوسف بم

أجريت هذه الدراسة لمعرفة أثر إضافة الخميرة المعقمة أو الحية علي مقاييس التخمر في الكرش. لذلك تم إجراء تجربتين باستخدام جهاز محاكاة الكرش المكون من وعاءين للتخمر يحتوي كل منهما علي سائل الكرش. وُضع في كل وعاء كيس نايلون يحتوي علي ١٥ جرام من العليقة المكونة من ٧.٥ جرام من المادة المالئة (سيلاج الحشائش وسيلاج الذرة) وكذلك ٧.٥ جرام من المادة المركزة هذا مع استبدال الأكياس كل ٤٨ ساعة لمدة ١٠ أيام. في التجربة الأولي احتوي وعاء التخمر الأول علي العليقة الضابطة (الخالية من الخميرة بنوعيها) والمكونة من ٧.٥ جرام من مركزات الطاقة بالإضافة إلي ٧.٥ جرام من المادة المالئة سالفة الذكر. في حين احتوي وعاء التخمر الثاني علي ٥ جرام من مركزات الطاقة وكذلك ٢.٥ جرام من مركزات البروتين المزودة بالخميرة المعقمة بالإضافة إلي ٧.٥ جرام من المادة المالئة. هذا وقد أستخدم الوعاء الأول كعليقه ضابطة في التجربة الثانية حيث احتوي علي ٧.٥ جرام من المادة المالئة و ٧.٥ جرام من مركزات الطاقة في حين احتوي الوعاء الثاني علي ٥ جرام من مركزات الطاقة وكذلك ٢.٥ جرام من مركزات البروتين المزودة بالخميرة المعقمة بالإضافة إلي الخميرة الحية بالاضافة الي ٧.٥ جرام من المادة المالئة. أظهرت النتائج أن إضافة مستنبت الخميرة أحدث تغييرات في مقاييس تخمر الكرش علي هيئة زيادة معنوية في تركيز حامض ألخليك مع زيادة رقمية في باقي الأحماض الطيارة الفردية وكذلك التركيز الكلي للأحماض. وذلك بالتوازي مع نقص معنوي في حامض ايزوفاليرك وكذلك حجم الغاز

الكلي الناتج من عملية التخمر، بينما أدت إضافة الخميرة الحية إلي نقص حجم الغاز الكلي ولم يكن لها أي اثر معنوي علي أي من مقاييس التخمر. مما سبق يتضح أن مستنبت الخميرة المعقمة وكذلك الخميرة الحية توثر علي مقاييس الكرش وقد كان هذا التغيير أكثر وضوحا مع الخميرة الحية تحت ظروف التجربة الحالية.

SUMMARY

The objective of this study is to determine whether an autoclaved yeast culture of *Saccharomyces cerevisiae* or live yeast has an effect on the parameters of ruminal fermentation. For this purpose, autoclaved yeast cultures and live yeast were tested simultaneously by the rumen simulation technique (Rusitec). Each fermentation vessel received daily 15 g feed bag consists of 7.5 g dried basal feed (5 g grass silage + 2.5 g corn silage) and 7.5 g concentrate pellets. Two experiments with two fermentation vessels were carried out. In exp 1 the 1st vessel (control) received 7.5 g energy concentrate in addition to 7.5 g dried basal feed. The second fermentation vessel (T1) received 5 g energy concentrate plus 2.5 g protein concentrate containing autoclaved yeast culture in addition 7.5 g basal feed. In exp 2 the vessel 1 received the same control diet as in experiment one, while vessel 2 (T2) received 5 g energy concentrate + 2.5 g of protein concentrate containing autoclaved yeast culture and live yeast in addition to 7.5 g basal feed. Yeast culture influenced the ruminal fermentation kinetics in terms of lower ($P<0.05$) gas volume and higher acetate (mol %), with a concomitant decrease in iso-valerate (mol %) in experiment 1, moreover, yeast culture numerically not statistically increase individual and total volatile fatty acids. in experiment 2, the living yeast cells decreased gas volume more than yeast culture but not alter any other rumen fermentation kinetics. In conclusion autoclaved yeast culture and living yeast cells had beneficial effects on rumen fermentation and this effect was more pronounced with live yeast culture.

***Key words**: Autoclaved yeast culture, live yeast, Rusitec, fermentation parameters*

INTRODUCTION

Addition of *Saccharomyces cerevisiae* cultures to ruminant diets improved the digestibility of dry matter, crude protein, and hemicellulose; increased ruminal bacterial numbers; decreased ruminal lactate concentrations; and increased milk production of cows in early lactation (Gomez-Alarcon *et al.,* 1990). *Saccharomyces cerevisiae* cultures provide soluble growth factors (i.e., organic acids, B vitamins, and amino acids) that are required by ruminal bacteria for growth on lactate (Nisbet and Martin, 1991). Dietary supplements of yeast culture, based on dried *Saccharomyces cerevisiae*, have been reported to increase productivity in ruminants, by a mechanism that results in an increased viable count of bacteria in the rumen which may lead to improved protein flow to the small intestine (Newbold 1995). Nisbet and Martin (1991) reported that *S. cerevisiae* stimulated the growth of the prominent lactic acid-utilizing rumen bacterium, *Selenomonas ruminantium*, in pure culture, apparently because dicarboxylic acids in the yeast stimulated lactate uptake by the bacterium. Moreover, Newbold *et al.* (1996) suggested two modes of action of yeast in stimulating rumen fermentation; the first, yeast respiratory activity protects anaerobic rumen bacteria from damage by oxgen, the second, yeast provides malic and other dicarboxylic acids which stimulate the growth of some rumen bacteria. They concluded that the stimulation of rumen bacteria by *S. cerevisiae* is at least partly dependent on its respiratory activity, and is not mediated by malic acid. Ruminal digestion of low quality feedstuffs provides the host volatile fatty acids and microbial protein to support energy requirements for sustenance, growth and work. Only about 10–35% of dietary energy consumed by the ruminant is conserved, however, improvements in digestive efficiency could improve ruminant animal production, with lowering input costs and undesired environmental impacts (Varga and Kolver, 1997). Ruminal methane production, for instance, results in the inefficient conversion of potentially energy-yielding substrates into a form that can not be conserved by the host. There have been many attempts to manipulate the rumen fermentation with the intention of increasing the production of propionate and reducing the production of methane. There are advantages in both directions, since the production of methane represents a direct loss of energy, while propionic acid is an important precursor for gluconeogenesis.

The rumen simulation technique (Rusitec) makes it possible to examine the direct effects of autoclaved yeast cultures or live yeast as additives in ruminant diets independent of the host ruminant. Therefore, the objective of this study was to investigate the effect of two types of yeast (autoclaved or live) on rumen fermentation parameters in vitro.

MATERIALS and METHODS

The experimental design is illustrated in Table 1. Two experiments were carried out using two Rusitec fermentation vessels described by Czerkawski and Breckenridge (1977). The incubation vessels was filled with rumen fluid. Rumen contents were taken from a rumen of fistulated cow that was maintained on a diet of grass hay and grain concentrates. Animals had free access to hay, water, and a vitamin-enriched salt lick. Each vessel was loaded with 2 nylon bags. The nylon bags (70×120 mm) had a pore size of 150 μm which is usually used for *in vitro* rumen fermentation studies (Öztürk, 2003). At the start of the trial, one bag was filled with 80 g of solid rumen contents to inoculate particle-associated microorganisms into the system and the other with the daily tested diets. The nylon bag with solid rumen contents was replaced after 24 h of incubation with a bag containing the diet. The feed bag was changed after 48 h so that 2 bags were always present. This gave a retention time of 48 h for feed. Bags were exchanged under anaerobic conditions using N2 to flush the incubation vessels. To maintain conditions as close to those of the *in vivo* rumen as possible, the incubation temperature was 39°C and rumen fluid turnover was simulated by modified artificial saliva. By moving the inner vessel up and down continuously rumen motility was simulated and exchange between the fluid and particle phases was completely done. In both experiments each vessel received 7.5 g dried basal feed (5 g grass silage + 2.5 g corn silage) and 7.5 g concentrate. In exp 1 the 1st vessel (control) received 7.5 g of energy concentrate (kombilac 16) beside 7.5 g of the basal feed, while the second fermentation vessel (T1) received a concentrate mixture consisting of 5 g energy concentrate (Kombilac 16) and 2.5 g protein concentrate containing 2.47% autoclaved yeast culture (Rumenac Prolactin) in addition to 7.5 g basal feed. In Exp. 2 vessel 1 received the same control diet as in experiment 1, however, vessel 2 received 5 g of energy concentrate plus 2.5 g protein concentrate containing autoclaved yeast (Rumenac Prolactin) in addition to a feed

additives containing live yeast (Rumex SC) beside the basal feed. The chemical composition of the experimental diets is presented in Table 2. An equilibration period of 5 days was allowed before the rumen fluid and the effluent samples were collected for the next 5 days (collection period) and repeated 3 times, and sequential samples of ruminal fluid were taken from inside the vessels before replacing the feed bags. Gas volume was determined daily by gas bag. The liquid flow through the vessels was maintained by continuous infusion of a buffer solution (pH 7.4) at the rate of 750 ml/day the composition of the buffer is shown in Table 3 according to McDougall's (1948). All additives were supported by Fixkraft® company (Enns, Austria) except Rumex SC was supported by Delacon company®, Austria.

Analytical procedures and samplings

Most of the analytical procedures used were as described by Czerkawski and Breckenridge (1977*)*. The pH and redox potential values were measured daily in each vessel at the time of feeding using a pH and redox electrodes (Typ 408, Mettler Toledo, Steinbach, Germany) connected to a Knick pH meter (digital pH meter 646, Knick, Berlin, Germany). Liquid effluent was collected daily and samples were taken for analyses of microbial particulate DM. Rumen fluid samples was mixed with oxalic acid (0.1 mol/l), sodium azide (40 mmol/l) and capronic acid (0.1 mmol/l) as internal standard, centrifuged and the supernatant was analyzed for short chain fatty acids (SCFAs) by gas chromatography (Agilent 6890 N GC) equipped with a 30 m x 530µm x 0.1µm capillary column with flame ionization detector according to the method described by Schafer, (1995). Rumen ammonia was analysed using test kits (Sigma – Aldrich) by spectrophotometer (Hitachi, U-3000, USA)

Statistical analyses

Statistical analyses were conducted with the Statistical Package for Social Science (SPSS for Windows Version 13; SPSS GmbH, Munich, Germany) to determine if the variables differed between groups. Differences between the means were compared by independent t-test. Probability values of $P<0.05$ were considered as significant. Values in tables are means ± SD.

RESULTS

Effects of yeast culture on ruminal fermentation *in vitro* are shown in Tables 4, 5, 6 and 7. **In experiment 1:** the addition of autoclaved yeast culture in the fermentation vessel significantly decreased the gas volume and the molar proportion of isovalerate volatile fatty acid in concomitant with significant increase in the molar proportion of ruminal acetate. In addition, there are numerical increases in the effluent microbial dry matter out put (particulate dry matter, PDM), the concentration of propionate, other individuals and the total daily volatile fatty acids (VFA) by the addition of autoclaved yeast culture to the fermentation vessels. The autoclaved yeast culture exhibit no significant ($P > 0.05$) impact on rumen pH, redox potential and ammonia concentration in experiment 1.

In experiment 2: a significant reduction in gas volume from vessel 2 receiving the living yeast cell in comparison with vessel receiving the control diet. In addition, there are numerical increases in the individual and total volatile fatty acids. No significant impact was noticed in experiment 2 on other rumen fermentation kinetics.

Table 1: Experimental design.

Experiment	Vessel 1	Vessel 2
1	7.5 g basal feed* (roughages) 7.5 Energy Concentrate (kombilac 16) (control)	7.5 g basal feed (roughages) 5 g Energy Concentrates 2.5 g protein concentrate containing autoclaved yeast culture (Ruminac prolactin (T1)
2	7.5 g basal feed (roughages) 7.5 Energy Concentrate(kombilac 16) (control)	7.5 g basal feed (roughages) 5 g Energy Concentrates 2.5 g protein concentrate containing autoclaved yeast culture (Ruminac prolactin + live yeast (Rumex SC) (T2)

* Basal feed = 5 g grass silage + 2.5 g corn silage
Ruminac prolactin contains autoclaved yeast culture (2.47 %)

Table 2: Chemical composition of the experimental diet (%, as fed basis)

Item	DM	OM	CP	EE	CF	NFE	Ash	GE[1]
Grass silage	53.1	47.3	9.5	1	11.9	24.9	5.8	10.138
Corn silage	35.4	34.1	2.3	1.5	6.6	23.7	1.3	6.656
Kombilac 16	87.6	82.7	16.9	3	5.3	57.5	4.9	14.857
Rumenac prolactin	90	85	20.3	2.8	5.2	56.7	5	15.369
Rumenac prolactin + Rumex SC	87.4	82.3	20.4	3.1	4.8	54	5.1	14.506

[1] GE KJ/g = Determined by bomb calorimeter
Kombilac 16= Energy concentrate contains 16 % CP and 7 MJ NEL, Rumenac prolactin= protein concentrate contains 20 % CP, 7.5 MJ NEL and yeast culture (2.47 %), Rumex SC= (natural feed additives) containing live yeast.

Table 3: Chemical composition of modified artificial saliva (RUSITEC buffer)

Ingredient	g/l	mmol/l
Sodium chloride (NaCl)	1.6380	28
Potassium chloride (KCl)	0.573	7.69
Calcium chloride ($CaCl_2$ $2H_2O$)	0.0323	0.22
Magnesium chloride (Mg Cl_2 6 H_2O)	0.128	0.63
Ammonium chloride (NH_4 Cl)	0.267	5.00
Disodium hydrogen phosphate (Na_2HPO4 12 H_2O)	3.58	10.00
Sodium Hydrogen phosphate ($NaH2PO4$ H_2O)	1.38	10.00
Sodium bicarbonate ($NaHCO_3$)	8.224	97.90

Table 4: Effect of autoclaved yeast culture on rumen fermentation parameters in Rusitec (Exp.1)

Items	Control	Autoclaved yeast culture	P
Ph	6.47 ± 0.08	6.45 ± 0.04	0.2
Redox potential (mv)	-286.5 ± 19	-281± 21	1.2
Gas volume (ml)	1263 ± 84[a]	1150 ± 77[b]	0.000
Ammonia (g/l)	0.20 ± 0.05	0.19 ± 0.06	0.8
Effluent PDM (g/l)	0.50 ± 0.06	0.71± 0.14	0.15

[a, b] Means within a row with different superscripts are significantly different (P <005).
PDM = particulate dry matter (microbial output)

Table 5: Effect of autoclaved yeast culture on rumen VFA and the molar % VFA (Exp. 1)

Acids	Control	Autoclaved yeast culture	P
Acetate (mmol/l)	19.4 ± 2.1	20.9± 4.6	0.18
Propionate (mmol/l)	7.8 ± 0.6	8.1 ±1.7	0.4
i-butyrate (mmol/l)	0.27 ± 0.03	0.3 ± 0.1	0.25
n-butyrate (mmol/l)	6.10 ± 0.6	6.4 ± 1.3	0.18
i-valerate (mmol/l)	1.7 ± 0.3	1.5 ± 0.3	0.08
n-valerate (mmol/l)	2.0 ± 0.4	2.1 ± 0.6	0.62
Total VFA (mmol/l)	37.1 ± 3.9	39.2 ± 8.4	0.18
	……mol %.....		
Acetate	52.0 ± 0.3 [a]	53.2 ± 0.5 [b]	0.001
Propionate	20.9 ± 0.9	20.7 ± 0.4	0.25
n-butyrate	16.4 ± 0.5	16.4 ± 0.2	0.15
i-butyrate	0.7 ± 0.1	0.7 ± 0.1	0.08
i-valerate	4.4 ± 0.5 [a]	3.7 ± 0.4 [b]	0.62
n-valerate	5.4 ± 0.5	5.3 ± 0.4	0.3

Table 6: Effect of live yeast on fermentation parameters (Exp. 2)

Items	Control	Live yeast	P
pH	6.51 ± 0.05	6.49 ± 0.08	0.3
Redox potential (mv)	-295.7 ± 25	-288.2 ± 16	0.08
Gas volume (ml)	1305 ± 52 [a]	1080 ± 81 [b]	0.02
Ammonia (g/l)	0.23 ± 0.03	0.17 ± 0.03	0.09
Effluent PDM (g/l)	0.51± 0.09	0.63 ± 0.09	0.08

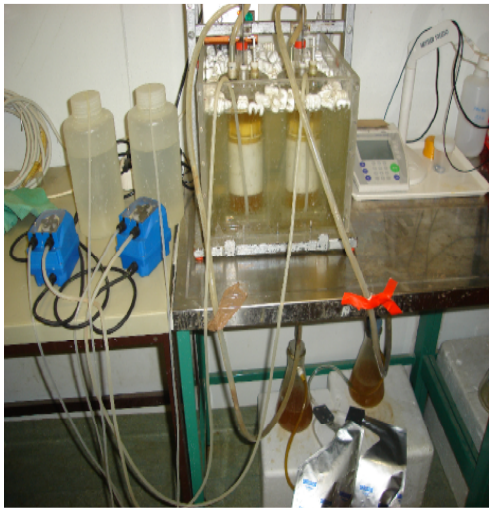
[a, b] Means within a row with different superscripts are significantly different (P <005).
PDM = particulate dry matter (microbial output)

Table 7: Effect of live yeast on rumen VFA and the molar % VFA (Exp. 2)

Acids	Control	Live yeast	P
Acetate (mmol/l)	19.1 ± 5.1	20.0 ± 3.1	0.4
Propionate (mmol/l)	8.8 ± 2.6	9.2 ± 1.6	0.51
i-butyrate (mmol/l)	0.3 ± 0.1	0.3 ± 0.1	0.09
n-butyrate (mmol/l)	5.5 ± 1.6	6.2 ± 0.9	0.54
i-valerate (mmol/l)	1.5 ± 0.6	1.73 ± 0.6	0.35
n-valerate (mmol/l)	2.3 ± 0.8	2.5 ± .5	0.22
Total VFA (mmol/l)	37.5 ± 10.7	40 ± 6.7	0.08
	……mol %.....		
Acetate	51.3 ± 1.39	50.1 ± 1.0	0.43
Propionate	23.4 ± 0.8	22.9 ± 0.6	0.2
n-butyrate	14.7 ± 0.9	15.6 ± 0.5	0.09
i-butyrate	0.8 ± 0.1	0.8 ± 0.01	0.67
i-valerate	3.8 ± 0.6	4.2 ± 0.8	0.32
n-valerate	6.0 ± 0.4	6.3 ± 0.5	0.24

A

B



C

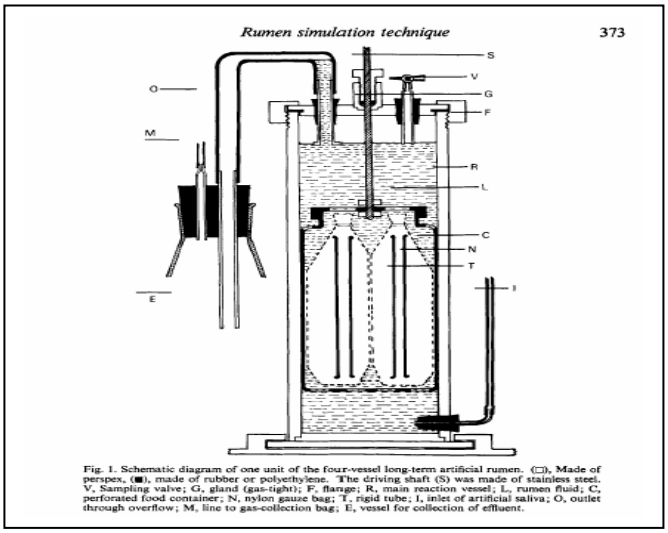


Fig. 1: A and B (Rusitec) Rumen Simulation technique design used in the experiment. C. Schematic diagram of one vessel of rusitec adapted from Czerkawski and Breckenridge (1977).

DISCUSSION

In animals fed high-energy diets, decreased lactic acid concentrations are associated with higher ruminal pH, and are characteristic of much more stable ruminal fermentation. These alterations in ruminal fermentations can be expected for improved digestion, and could also be reflected in improved intake. The ability of yeast to prevent the accumulation of lactic acid in the rumen suggests a role for viable yeast in overcoming ruminal dysfunctions associated with the use of high energy diets used in both high-producing dairy and fast-growing beef cattle. The significant increase in acetate and numerical increase in some individual and total VFA by the addition of yeast culture was in agreement with results of Callaway and Martin, (1997) and Oeztuerk, (2009). They reported that yeast culture increased the concentrations of acetate and total volatile fatty acids that produced by *Sel. ruminantium* HD4 and increased the concentrations of propionate and total volatile fatty acids that produced by *Sel. ruminantium* H18. Callaway and Martin, (1997) suggested that, yeast culture provides soluble growth factors (i.e., organic acids, B vitamins, and amino acids) which stimulate growth of ruminal bacteria that utilize lactate and digest cellulose. So, dietary supplementation with yeast cultures that are high in these growth factors may improve ruminal fermentation. Moreover, Wallace and Newbold (1992) concluded that the production benefits seen when yeast culture is added to the diet arise from changes in the stoichiometry of VFA formation. The increase in the proportion of acetate was also observed by the experiment of Mutsvangwa *et al.* (1992) with yeast culture. The positive effect of living yeast cell in experiment 2 was in agreement with that of Dawson *et al.* (1990) and Callaway and Martin, (1997) who reported that heat inactivated yeast culture preparations have no effect on ruminal bacterial growth, suggesting that live yeast cells are necessary for the stimulation of growth of lactate utilizing bacteria *Sel. ruminantium*.

The decrease in gas production which is mainly methane is a promising result, because methane emissions represent losses of up to 15% of gross energy intake for forage-fed cattle and losses of 2–4% for cattle consuming diets rich in readily fermentable substates (Johnson and Johnson, 1995). There have been many attempts to manipulate the rumen fermentation with the intention of increasing the production of propionate and reducing the production of methane. There are advantages in both directions, since the production of methane

represents a direct loss of energy while, propionic acid is an important precursor for gluconeogenesis. It can thus have a protein sparing role since amino acids are the other main source of glucose precursors (Leng, 1970).

In the current study, many of the changes associated with the addition of yeast cultures were marginal and often not statistically significant. This makes interpretation difficult, In conclusion, these results indicated that, the addition of either autoclaved or live yeast cultures stimulated ruminal fermentation and this effect was more pronounced with live yeast culture.

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