Interaction between Live Yeast and Dietary Rumen Degradable Protein Level: Effects on Diet Utilization in Early-Lactating Dairy Cows

Christine Julien, Jean Philippe Marden, Eric Auclair, Raymond Moncoulon, Laurent Cauquil, Jean-Louis Peyraud, Corine Bayourthe

Agricultural Sciences, 2015, 6, 1-13
Published Online January 2015 in SciRes. http://www.scirp.org/journal/as
http://dx.doi.org/10.4236/as.2015.61001

Interaction between Live Yeast and Dietary Rumen Degradable Protein Level: Effects on Diet Utilization in Early-Lactating Dairy Cows

Christine Julien[1,2,3*], Jean Philippe Marden[4], Eric Auclair[4], Raymond Moncoulon[1,2,3], Laurent Cauquil[1,2,3], Jean Louis Peyraud[5], Corine Bayourthe[1,2,3]

[1]Université de Toulouse INPT ENSAT, UMR1388 Génétique, Physiologie et Systèmes d'Elevage, Castanet-Tolosan, France
[2]INRA, UMR1388 Génétique, Physiologie et Systèmes d'Elevage, Castanet-Tolosan, France
[3]Université de Toulouse INPT ENVT, UMR1388 Génétique, Physiologie et Systèmes d'Elevage, Toulouse, France
[4]Lesaffre Feed Additives, Marcq-en-Baroeul, France
[5]INRA, UMR1348 PEGASE, F-35590 Saint-Gilles, France
Email: *c.julien@ensat.fr

Received 1 November 2014; revised 27 November 2014; accepted 3 December 2014

Abstract

Four early lactating Holstein cows were used to study the effect of live yeast (LY, Actisaf® CNCM I-4407, Lesaffre Feed Additives, Marcq en Baroeul, France) supplementation on diet digestive utilization of dairy cows receiving concentrated corn silage-based diets with two rumen-degradable protein (RDP) levels. For a 33 d period, cows were fed a total mixed ration (TMR) containing an adequate level (AL) of RDP or a low level (LL, 30% below AL) by using soybean meal or tanned soybean meal, respectively: for 21 d with no LY addition followed by 12 d during which LY was added to the diet. The pH and redox potential (E_h) were recorded and ruminal fluid samples were collected over 3 consecutive days. Feces were collected individually over 48 h and individual dry matter intake (DMI) was measured for determining apparent nutrient digestibility. The effective degradability of individual feed ingredients composing both diets was evaluated with nylon bags technique. Structure of the ruminal bacterial community was studied and diversity index was calculated. Digestibility of organic matter (OM) and crude protein (CP) were lower for LL than those for AL. With LY, digestibility of OM and CP was increased: +2.4 and +0.8 points, for AL, and +3.7 and +5.9 points for LL, respectively. Live yeast reduced dietary N ruminal degradation with both AL and LL. Ruminal pH and E_h were lower with AL compared to LL: 5.95 and −167 mV vs. 6.13 and −144 mV. Live yeast increased ruminal total volatile fatty acids (VFA) (+8.6%), C2 (+10%), and C4 (+35%) contents for LL and decreased that of C3 (−9.8%) for AL. Neither the structure of bacterial

How to cite this paper: Julien, C., Marden, J.P., Auclair, E., Moncoulon, R., Cauquil, L., Peyraud, J.L. and Bayourthe, C. (2015) Interaction between Live Yeast and Dietary Rumen Degradable Protein Level: Effects on Diet Utilization in Early-Lactating Dairy Cows. *Agricultural Sciences*, **6**, 1-13. http://dx.doi.org/10.4236/as.2015.61001

populations of the rumen nor the diversity index (Shannon) was altered by treatments. Those results suggested a specific interest in using LY in RDP deficient diets for early lactating cows.

Keywords

Dairy Cow, Live Yeast, Nitrogen Digestion, Ruminally Degradable Protein

1. Introduction

Early lactation high-producing dairy cow nutrition strategies aim at providing adequate energy and rumen undegraded protein (RUP) to support high requirements regarding milk production increase and because, most of the time, cows are in negative energy balance. On one hand, absorbed protein requirements implied that more dietary protein escapes rumen degradation with the risk that ruminal ammonia concentration is insufficient to support rumen microbial crude protein (CP) production. This can cause a depression in fiber degradation, and reduce dry matter intake (DMI) and energy supply to the animal. On the other hand, it is necessary to increase the proportion of grain to increase energy content of the diet. This strategy resulting in an increase in non structural carbohydrates (NSC) supply, can favor the appearance of ruminal acidosis precipitating depressed fiber digestion [1]. An imbalance in NSC and rumen degradable protein (RDP) may be responsible for uncoupled fermentation and consequent reduction in ruminal microbial growth and/or activity. During this critical period, many alternatives have been evaluated to improve digestive processes in the rumen and increase feed efficiency.

Many studies put forward the relevant use of live yeast (LY) as a feed additive for early lactating dairy cows by, most of the time, questioning LY effect on energetic metabolism. Inclusion of LY has been shown to stabilize the rumen environment through higher pH values and enhance fiber digestion [2]-[4]. These key results certainly explain why the impact of a LY supplementation has been studied primarily on energetic metabolism, in relation with the dietary forage: concentrates ratio [5]-[10]. The consensus was that the effects of LY were enhanced when animals consumed a high concentrated diet or during an abrupt dietary transition, as also highlighted by Chaucheyras-Durand *et al.* [11]. However, regarding determinations of ruminal ammonia nitrogen (N), some of these works also have been conducted to often consider a potential effect of LY on N metabolism, more specifically on protein degradation and/or microbial proteosynthesis.

To our knowledge, no study has already questioned the interaction between dietary N level, in terms of RDP to RUP ratio, and LY supplementation in dairy cow. Sniffen *et al.* [12] simply pointed out the fact that the diet balance between RDP and RUP could be a key factor in the response to LY supplementation. In early lactation dairy goats, Giger-Reverdin *et al.* [13] tested the effect of LY addition on DMI, milk production and blood parameters at two levels of RDP intake (10.8% and 7.7%, on a DM basis) and at a constant RDP to RUP ratio. These authors concluded that LY effect depended on the N level of feed. It was therefore necessary to consider the LY and dietary RDP content interaction effect on lactating dairy cow N utilization and to investigate its potential effect when incorporated in not correctly RDP and RUP balanced diet. In this context, the present study aimed at questioning the effect of LY supplementation on diet digestive utilization of dairy cows receiving concentrated corn silage-based diets with two different RDP to RUP ratios.

2. Materials and Methods

2.1. Animals, Experimental Design, and Diets

Four ruminally cannulated early (46 ± 20 days in milk) lactating Holstein dairy cows (mean milk production of 35.6 ± 2.4 kg/d) were involved in a cross-over experimental design. Cannulation techniques provided for humane treatment of cows adhered to locally approved procedures and were similar to those described by Streeter *et al.* [14]. Animals were housed in individual tie stalls throughout the experiment with free access to water. Both experimental diets were balanced to meet energy and CP requirements for maintenance and production of dairy cows in early lactation, producing 35 kg/d of milk with 3.0% of fat and 3.0% of protein [15] at a level intake of 20 kg DM. Cows were fed a diet (AL) balanced to meet RDP requirements [15] or a diet (LL) to contain 30% less RDP. Soybean meal (SBM) and tanned soybean meal (TSBM) were the feed ingredients chosen as the

primary source of proteins to achieve formulation of these two experimental diets (**Table 1**). Both experimental diets included recommended level of RUP and were fed as total mixed ration (TMR) with two equal distributions, at 0900 and 1700 h.

Each of the 2 experimental period consisted of 33 d. Cows were fed control diets for 21 d with no live yeast (LY) addition (14 d of adaptation and 7 d of sampling and measurements period), followed by 12 d with LY addition in which 5 d were allowed for adaptation to LY and 7 d of sampling and measurements period. Cows received randomly one of the two diets (AL or LL) during the first period, and the other one during the second period. The recommended LY dose of 5 g/cow/d (10^{10} cfu/g of DM, Actisaf® CNCM I-4407, Lesaffre Feed Additives, Marcq en Baroeul, France) was top-dressed on the TMR. Dairy cows were milked twice a day at 0700 and 1700 h and weighted on two consecutive days before and after LY supplementation of each experimental period.

2.2. Measurements and Sampling

2.2.1. Ruminal Physicochemical Measurements and Fermentative Parameters

Measurements of pH and redox potential (E_h) were realized over 3 consecutive days (d 15 to d 17 and d 27 to d 29) using the *ex vivo* method described by Julien *et al.* [16]. For each cow, ruminal pH and E_h were recorded hourly over a 9-h period from 1 h before to 8 h after the morning meal (T_{-1} to T_{+8}). On the same days, for each treatment, two 10-mL ruminal fluid samples were collected at T_0, T_{+1}, T_{+2}, T_{+4}, T_{+6} and T_{+8}. Samples were preserved by the addition of 1 mL of mercuric chloride (2% wt/vol) for subsequent volatile fatty acids (VFA), lactic acid and ammonia-N (NH_3-N) determinations.

2.2.2. Apparent Total Tract Digestibility of Diet

Feces were collected individually and quantitatively during 48 h, starting at 0800 h of d 20 and d 32, and finishing at 0800 h of d 22 and d 34 of each experimental period. Collection was performed manually, over the whole

Table 1. Ingredients, chemical composition and nutritional value of the total mixed diets fed to lactating dairy cow.

	AL[1]	LL
Ingredients, % DM		
Corn silage	62.5	62.6
Wheat	18.0	18.0
Soybean meal	17.6	-
Tanned soybean meal	-	17.5
Minerals and vitamins	1.9	1.9
Chemical composition, % DM		
Organic matter	94.5	94.6
Crude protein	15.7	15.6
Neutral detergent fiber	34.1	34.8
Acid detergent fiber	18.0	17.8
Starch	29.1	29.1
Non structural carbohydrate[2]	42.3	41.6
Nutritional value[3]		
NE_L, Mcal/kg DM	1.67	1.65
RDP, % on DM basis	10.6	7.5
RUP, % on DM basis	5.1	8.1

[1]AL = adequate level of rumen degradable protein; LL = low level of rumen degradable protein. [2]Calculated as [100 − (CP + NDF + ash + fat)]. [3]NE_L = net energy for lactation; RDP = rumen degradable protein; RUP = rumen undegradable protein.

48 h, into plastic barrels. The collected feces were weighed daily. After thoroughly mixing, two subsamples (approximately 1 kg) were taken: one immediately dried at 104˚C for DM determination and the second frozen at –20˚C for subsequent organic matter (OM) and CP determinations. Feed intake and refusals were recorded daily on d 19 to 21 and on d 31 to 33 to determine individual DMI of the cows. Representative samples of dietary ingredient were taken each day then analyzed for DM, OM and CP. Apparent nutrient digestibilities of the diet were calculated from the different measurements.

2.2.3. *In Situ* Incubations

Individual feed ingredients composing both diets were dried at 60˚C and ground through 1-mm screen. Then, samples (3 g) of each were weighed into small (7.0 × 11.0 cm) heat-sealed nylon bags (Buisine, Clermont de l'Oise, France; mean pore size of 51 µm). Fifteen bags per feedstuff per cow were introduced into the ventral sac of the rumen immediately before the morning meal and anchored by a nylon cord string to the cap of the ruminal cannula. Three bags per incubation time were removed from the rumen after 2, 4, 8, 16 and 48 h for wheat, SM and TSM, and after 2, 8, 16, 48 and 72 h for maize silage. Upon removal, they were briefly rinsed under cold tap water to eliminate surface debris and machine-washed (2 × 5 min) to remove rumen fluid. Then nylon bags were dried at 60˚C for 48 h in a forced air oven. Bags were wheighed and residues analyzed for DM, OM and CP.

For each incubation time ruminal disappearance (%) of nutrients was calculated from the proportion remaining in the bags. Data were fitted to the nonlinear regression equation of Orskov and McDonald [17]: $Dis(t) = a + b(1 - e^{-ct})$ where Dis is percentage disappearance of nutrient at time t; a represents the soluble fraction, b the less rapidly degradable fraction which disappears at the constant fractional rate c per time t. The nonlinear parameters a, b and c were estimated by an iterative least squares procedure, with the constraint that $a + b \leq 100$. The constants (a, b, c) were later used to calculate the theoretical effective degradability (ED) of nutrient as follows: $ED = a + (b \times c)/(c + k)$ where k is the rumen outflow rate. The outflow rate was calculated for each cow at each experimental period by using equation of NRC [15] taking into account individual DMI and percentage of concentrate in the diet.

2.2.4. Purine Derivatives in Urine

During the two consecutive days of feces collection, a spot sample of urine (500 mL) was made once a day around milking hour after manual stimulation. A 10 mL-aliquot was immediately mixed with 90 mL of 0.036 N H_2SO_4 and stored at −20˚C pending allantoin, creatinine and uric acid determinations.

2.2.5. Structure of Rumen Bacterial Community

On d 17 and d 29 of each experimental period, 1 L of ruminal content was collected at T_{+4} then strained through a metal sieve (1.6 mm mesh). Approximalely 200 mg of strained ruminal fluid filtrate were precisely weighted and kept in a vial warranted without any DNAase or RNAase and then frozen at −80˚C for subsequent analysis based on molecular biology techniques.

2.3. Analytical Methods

Dry matter and OM content of samples were determined by oven drying at 104˚C for 24 h (48 h for feces) and by ashing at 550˚C for 12 h, respectively. Crude protein was analyzed using a Leco FP-428 Nitrogen Combustion Analyzer (Leco, St. Joseph, MI, USA). The concentrations of VFA were determined using the gas chromatographic method of Playne [18], modified by Marden *et al.* [3]. The determination of NH_3-N was based on the modified Berthelot reaction with the Skalar Method [19]-[21] on the separated liquid phase of ruminal samples centrifuged at 4000 × g for 20 min. Total lactic acid was determined enzymatically using a commercial kit (D-lactic acid/L-Lactic acid, UV method, Cat. No. 11 112 821 035, Roche). Purine derivatives (PD) in urine (allantoin, uric acid and creatinine) were determined from the adapted method from George *et al.* [22] by High Performance Liquid Chromatography (HPLC). Just before analysis, the urine samples were diluted tenfold with water after adjusting the pH to 7 using 0.01 N sodium hydroxide and 0.01 N sulphuric acid. Then 100 µL was mixed with 900 µL of ultrapure water. The quantitative HPLC separations were performed at a temperature of 25˚C (controlled by an oven) on a Phenomenex Synergi Hydro-RP C18 (250 mm × 4 mm I.D., 4 µm particule size). The mobile phase was 10 mM potassium dihydrogen phosphate solution (pH 4.7). The flow rate was 1 mL/min and the absorbance detector was set at 220 nm. Compound peaks were identified by the retention times and quantified by comparison of the peak areas of the samples with those of authentic standards on a 20 µL in-

jection. Total DNA from about 0.2 g of template was extracted with QIAamp® DNA Stool Mini kit (Qiagem S. A., Courtaboeuf, France). The V3 region of 16S rRNA bacterial genes was amplified by PCR using the primers w49 and 5'-6FAM-labeled w34 [23] [24]. Amplicons were subjected to capillary electrophoresis single-strand conformation polymorphism (CE-SSCP) as described by Michelland *et al.* [25]. The CE-SSCP profiles were aligned, normalized and Shannon diversity index and Richness were estimated using StatFingerprints program version 1.3 [26] running on R version 2.7.2 [27].

2.4. Calculations

The E_h yields only a rough indicator of the reducing power in a given milieu because it is highly dependent upon the effects of pH. To give a better appreciation of absolute reducing power, Clark's Exponent (rH) was calculated by integrating both pH and E_h values in the Nernst's equation: $rH = E_h\ (mV)/30 + 2\ pH$ [28]. Because of the diet being TMR, the assumption that the centesimal composition of the diet ingested by the animal was identical to that of a ration has been made. Potential ruminal degradation (PotRD) of OM and N of the diet distributed to each animal was calculated from the ED of each feed ingredients composing the diet measured on each cow in each period, with or without LY supplementation and from individual DMI. The amount of OM and N going out of the rumen was considered equivalent to the amount available in the duodenum. Total tract digestibility of the diet being measured, the OM and N post-ruminal disparition of the diet was estimated as follows:

Post-ruminal disparition = [Intake × (1 − PotRD) − Fecal excretion]/[Intake × (1 − PotRD)]

Urine volumes used to compute daily excretion of allantoin and uric acid from spot urine samples analysis were estimated: Body Weight × 29/creatinine concentration (mg/L) [29]. Total PD excretion was the sum of allantoin and uric acid excreted in urine.

2.5. Statistics

All statistical analysis was conducted using R software (2009). Sources of variation included design effects of period, time and fixed treatment effects of dietary RDP and LY addition. Cow was included as a random effect. All statistical analyses concerning microbiota study were carried out using StatFingerprints Package [26] with R (2.7.2 Version). An analysis of multiple variance 50 - 50 MANOVA was performed to study the structure of the bacterial communities taking into account diet and LY effects. Because the interaction between diet and LY was not significative, it was eliminated from the model. Difference between both diets was tested by unparametric test of Mann and Whitney. Diversity index and richness values were compared by means of univariate analysis including diet, LY and LY × diet interaction. Correlations between quantitative variables were calculated following Pearson. Multivariate correlations between quantitative variable and community structure were calculated by means of Statfingerprint package of R [26]. Differences were considered significant at $P \leq 0.05$ and trends were discussed at $0.05 < P \leq 0.10$. Differences between treatment effects were assessed by pairwise comparisons (Tukey's test).

3. Results

3.1. Ruminal Physicochemical Measurements and Fermentative Parameters

Ruminal pH, E_h and rH were significantly lower for HL compared to values recorded for LL: on average 5.95, −167 mV and 6.35 vs. 6.13, −144 mV and 7.47, respectively (**Table 2**). Ruminal pH did not differ significantly with LY supplementation, regardless of the level of RDP.

Regarding ruminal fermentative parameters, levels of IC5 were higher ($P = 0.0002$) and concentrations of NH_3-N were 2.3 times lower ($P < 0.0001$) in LL compared with HL. Ruminal lactic acid content tended ($P = 0.114$) to be different between HL and LL (5.45 mM and 4.55 mM, respectively), but was not influenced by the addition of LY. There was an interaction effect between LY and diet: LY supplementation induced a significant increase of ruminal total VFA (+8.5%), C2 (+10%), IC4 (+17.8%) and C4 (+35%) contents for LL but not for HL. Live yeast significantly decreased C3 ruminal content (−9.8%) whatever was the diet.

3.2. *In Situ* Ruminal Degradation of Dietary Ingredients

Live yeast had no significant effect on ruminal degradation parameters of OM and CP for SBM (**Table 3**). On

Table 2. Effect of live yeast and dietary RDP level on ruminal physicochemical and fermentative parameters.

	AL[1]		LL			*P*[3]		
	−LY	+LY	−LY	+LY	SEM[2]	D	LY	D × LY
Physicochemical parameters								
pH	5.95[a]	5.95[a]	6.19[b]	6.07[b]	0.02	<0.0001	0.27	0.15
E_h[4], mV	−166[a]	−167[a]	−147[b]	−140[b]	1.7	<0.0001	0.72	0.0002
rH[4]	6.36[a]	6.33[a]	7.48[b]	7.46[b]	0.07	<0.0001	0.88	0.001
Fermentative parameters								
Total VFA, mM	94.3[b]	89.6[ab]	84.1[a]	91.3[b]	1.4	0.006	0.75	0.005
C2, mM	49.6[a]	48.9[a]	48.7[a]	53.6[b]	0.68	0.24	0.13	0.012
C3, mM	28.7c	25.9[b]	18.5[a]	16.7[a]	0.56	<0.0001	0.0001	0.77
C4, mM	9.9[a]	9.8[a]	12.2[b]	16.5c	0.36	<0.0001	<0.0001	<0.0001
C2/C3	1.7[a]	2.0[b]	2.9c	3.3c	0.06	<0.0001	0.0001	0.33
C5, mM	4.0[b]	3.0[a]	2.6[a]	2.4[a]	0.11	<0.0001	0.001	0.006
IC4, mM	0.71[b]	0.66[b]	0.56[a]	0.66[b]	0.01	<0.0001	0.19	0.0009
IC5, mM	1.2[a]	1.3[a]	1.4[b]	1.3[b]	0.04	0.0002	0.71	0.60
NH_3-N, mg/L	81.2	81.6	33.1	37.9	3.3	<0.0001	0.99	0.53
Lactic ac.[5], mM	6.0	4.9	4.3	4.8	0.67	0.11	0.91	0.80

[1]AL = adequate level of rumen degradable protein; LL = low level of rumen degradable protein; −LY = without live yeast; +LY = 5 g of live yeast per cow and per day. [2]SEM = standard error of the mean. [3]D = diet effect; LY = live yeast effect; D × LY = diet × live yeast interaction effect. [4]E_h = redox potential; rH = E_h (mV)/30 + 2 pH. [5]Mean lactic acid concentration of ruminal fluid sampled at 1-h and 2-h post-feeding.

Table 3. Effect of live yeast and dietary RDP level on the rumen degradation characteristics of the OM and CP in soybean meal and tanned soybean meal.

		−LY	+LY	SEM[2]	*P*[3]
Soybean meal in AL[1] diet					
Organic matter	a[4]	22.7	29.8	3.0	0.31
	b[4]	71.4	62.6	3.2	0.22
	c[4]	7.9	6.4	0.78	0.45
	ED[4]	61.6	63.1	1.3	0.52
Crude protein	a	9.6	18.0	2.8	0.18
	b	84.8	73.0	3.7	0.06
	c	7.7	6.3	0.49	0.44
	ED	54.9	55.8	2.3	0.42
Tanned soybean meal in LL diet					
Organic matter	a	28.7	26.4	0.99	0.42
	b	37.7	41.8	2.7	0.50
	c	2.2	3.8	0.50	0.08
	ED	37.8	41.5	1.1	0.11
Crude protein	a	13.3	16.0	1.1	0.28
	b	35.7	41.2	4.9	0.63
	c	1.1	2.8	0.40	0.02
	ED	17.9	28.1	2.1	0.007

[1]AL = adequate level of rumen degradable protein; LL = low level of rumen degradable protein; −LY = without live yeast; +LY = 5 g of live yeast per cow and per day. [2]SEM = standard error of the mean. [3]LY = live yeast effect. [4]a = slowly degradable fraction (%); b = rapidly degradable fraction (%); c = rate of degradation of the b fraction (%/h); ED = effective degradability (%).

the contrary, EDCP of TSBM was significantly increased (+10 points) in presence of LY and the rate of degradation of the b fraction was twofold higher ($P = 0.025$): 1.1%/h to 2.8%/h.

3.3. Digestibility of the Diet

Results of the digestibility trial were presented in **Table 4**. Daily intake of OM and nitrogen (**N**) did not differ between diets, averaging 19 kg of OM and 534 g of N. The amount of N excreted in the feces was significantly lower for HL than for LL: respectively 128.5 and 232.9 g/d. Total tract digestibility of OM and CP were significantly higher for HL than LL: 69.5% vs. 66.3% and 75.1% vs. 57.9%, respectively. Potential ruminal degradation of OM was significantly lower for LL than HL (56.3% vs. 62.4%). Amount of OM going out of the rumen was significantly higher for LL than for HL: 8.4 vs. 7.3 kg/d. The amount of available duodenal N was on average higher ($P < 0.0001$) for LL (347.8 g/d) than for HL (238.6 g/d). Post-ruminal disparition of OM was higher for LL (21.6%) than HL (18.2%).

With LY supplementation, fecal OM and N excretion was reduced respectively by 19% and 20% for LL. Total tract digestibility of dietary OM and CP were significantly increased with LY supplementation: respectively +2.4 and +0.8 for HL, and +3.7 and +5.9 for LL. Potential ruminal degradation of OM was significantly reduced by 0.5 and 1.3 points in presence of LY, for HL and LL respectively. Potential ruminal degradation of dietary N significantly decreased with LY supplementation for both HL and LL by −0.6 points and 5.2 points, respectively.

Table 4. Effect of live yeast and dietary RDP level on OM and nitrogen digestion in lactating dairy cow.

	AL[1]		LL			*P*[3]		
	−LY	+LY	−LY	+LY	SEM[2]	D	LY	D × LY
Measured parameters								
Intake								
OM, kg/d	18.5	19.8	19.9	18.1	0.61	0.44	0.55	0.14
N, g/d	520.8	561.4	549.3	504.7	16.8	0.95	0.73	0.09
Fecal excretion								
OM, kg/d	5.6	5.5	6.7	5.4	0.20	0.003	0.007	0.09
N, g/d	128.5[a]	135.2[a]	232.9[c]	185.9[b]	11.2	<0.0001	0.03	0.03
Total tract digestibility								
OM, %	69.5	71.9	66.3	70.0	0.58	0.005	0.003	0.66
N, %	75.1	75.9	57.9	63.8	1.6	<0.0001	0.01	0.15
Estimated parameters								
Potential ruminal degradation								
OM, % of OM intake	62.6[c]	62.1[c]	56.9[a]	55.6[b]	0.75	<0.0001	0.004	0.03
N, % of N intake	56.2	55.6	36.0	30.8	2.2	<0.0001	0.001	0.25
Amount going out of the rumen[4]								
OM, kg/d	7.0	7.6	8.7	8.1	0.28	0.0001	0.87	0.39
N, g/d	228.1	249.1	350.5	345.0	13.4	<0.0001	0.29	0.13
Post-ruminal disparition[5]								
OM, %	18.2	26.0	21.6	32.4	1.5	0.05	0.001	0.43
N, %	42.3	45.8	39.7	47.3	1.6	0.21	0.02	0.96

[1]AL = adequate level of rumen degradable protein; LL = low level of rumen degradable protein; −LY = without live yeast; +LY = 5 g of live yeast per cow and per day. [2]SEM = standard error of the mean. [3]D = diet effect; LY = live yeast effect; D × LY = diet × live yeast interaction effect. [4]Taking into account only dietary OM and N. [5]Expressed as % of OM or N amount going out of the rumen. Different superscripted letters in each row indicate significant differences ($P < 0.05$).

The amount of N going out of the rumen did not differ with LY supplementation whatever was the diet. Post-ruminal disparition of OM and N was significantly increased by LY supplementation for both diets: respectively, +7.8 and +3.5 points for HL, and +10.8 and +7.6 points for LL.

3.4. Urinary Purine Derivatives

Total urinary PD excretion tended (P = 0.075) to be lower for LL than for HL: 85.5 vs. 90.0 mg/d (**Table 5**). There was a tendency (P = 0.089) for a LY × diet effect: LY tended to decrease urinary PD excretion (−45%) for LL but not for HL and specifically allantoine excretion (−46%), which represented, on average, 88% of total PD excretion whatever was the diet.

3.5. Ruminal Microbial Communities

The structure of the bacterial community was different between HL and LL (50-50 MANOVA, P = 0.03) but not altered by the addition of LY (50-50 MANOVA, P = 0.77). The mean profiles obtained by CE-SSCP for each diet differ by 29% depending on the iterative test of Mann and Whitney (data not shown). Addition of LY to the diet did alter neither richness nor diversity index of ruminal bacterial community (**Table 6**).

Richness of the bacterial community for HL was significantly higher than for LL: respectively 32.4 and 27.9. The diversity index of the ruminal bacterial community for LL was 10% lower than for HL (P = 0.032).

Richness of molecular fingerprints of bacterial communities was correlated (**Table 7**) with ruminal NH_3-N content (P = 0.031), C3 (P < 0.001), C4 (P = 0.007), C2:C3 ratio (P < 0.001) and rH (r^2 = 0.35, P = 0.025). Diversity index and CE-SSCP profiles were significantly correlated with C3, C4, and the C2:C3 ratio, and diversity index tended (P = 0.061) to be correlated with ruminal NH_3-N.

4. Discussion

In the present study, diets were formulated to have a higher starch content compared to the threshold value of 25% on a DM basis recommended by Sauvant *et al.* [30] to avoid ruminal latent acidosis. Consequently, both diets could be suspected to induce a ruminal acidotic state. Over the 8-hour period of post prandial measurements, ruminal pH of cows fed AL remained below 6.0 for 6 hours, with 4 hours less than 5.8, while it remained below 6.0 for 4 hours but never go below 5.8 for cows fed LL. So, only AL induced ruminal pH values reflecting the appearance of latent acidosis, as defined by Sauvant *et al.* [31]. The more pronounced effect on the dynamics of pH with AL than LL could be explain by different rapidly degradable DM (RDM, %) and degradable OM

Table 5. Effect of live yeast and dietary RDP level on urinary purine derivatives excretion.

	AL[1]		LL			P[3]		
	−LY	+LY	−LY	+LY	SEM[2]	D	LY	D × LY
Allantoine, mg/d	79.0	83.5	76.2	41.3	5.81	0.08	0.14	0.08
Uric acid, mg/d	11.0	10.7	9.3	5.6	6.47	0.05	0.17	0.25
PD[4], mg/d	90.0	94.1	85.5	46.9	0.78	0.07	0.14	0.08

[1]AL = adequate level of rumen degradable protein; LL = low level of rumen degradable protein; −LY = without live yeast; +LY = 5 g of live yeast per cow and per day. [2]SEM = standard error of the mean. [3]D = diet effect; LY = live yeast effect; D × LY = diet × live yeast interaction effect. [4]PD = Allantoin (mg/d) + Uric acid (mg/d).

Table 6. Effect of live yeast and dietary RDP level on richness and diversity index of rumen bacterial community.

	AL[1]		LL			P[3]		
	−LY	+LY	−LY	+LY	SEM[2]	D	LY	D × LY
Richness	32.5	32.3	28.0	27.7	0.78	0.003	0.81	0.93
Diversity index	6.65	6.68	5.99	5.98	0.16	0.03	0.83	0.84

[1]AL = adequate level of rumen degradable protein; LL = low level of rumen degradable protein; −LY = without live yeast; +LY = 5 g of live yeast per cow and per day. [2]SEM = standard error of the mean. [3]D = diet effect; LY = live yeast effect; D × LY = diet × live yeast interaction effect.

Table 7. Correlations between richness, Shannon diversity index or capillary electrophoresis single-strand conformation polymorphism profiles and ruminal parameters.

	Richness		Diversity		CE-SSCP profiles	
Parameters[1]	Pearson r^2	*P*	Pearson r^2	*P*	Pearson r^2	*P*
pH	0.18	0.13	0.07	0.37	0.12	0.11
E_h, mV	0.19	0.12	0.02	0.66	0.04	0.51
rH	0.35	0.02	0.06	0.41	0.08	0.16
Total VFA, mM	0.06	0.38	0.08	0.33	0.08	0.50
C2, mM	0.15	0.16	0.07	0.36	0.08	0.27
C3, mM	0.67	<0.001	0.42	0.01	0.31	<0.001
C4, mM	0.47	0.007	0.33	0.03	0.23	0.01
C2/C3	0.78	<0.001	0.52	0.004	0.34	<0.001
NH_3-N, mg/L	0.33	0.03	0.26	0.06	0.13	0.17

[1]E_h = redox potential; rH = Clark's Exponent.

(DOM, g/kg DM) contents, considered as accurate predictors of the response of ruminal pH [32]. Actually, by using analyzed chemical composition and *in situ* values of the feed ingredients, concentrates from AL diet provided greater amounts of RDM and DOM than LL: respectively 21.2% and 375 g/kg DM vs. 17.3% and 324 g/kg DM. However for both diet, the concentrate DOM content remained below the threshold value of 400 g/kg DM, recommended to prevent a drastic pH fall with high-starch diets [32]. This also supports that cows fed AL and LL were prevented from the appartition of acute ruminal acidosis (**Table 2**).

Nethertheless, LL implied a depleted fermentative activity compared to AL as shown by total VFA ruminal contents which differed significantly: the higher total VFA content observed with AL (94.3 vs. 84.1 mM) was caracterized by a lower acetate to propionate ratio (1.7 vs. 2.9). Propionic ruminal metabolic pathways were much more privileged when SBM was included in the diet than TSBM, leading to a significantly lower ruminal pH with AL than LL (5.95 and 6.13, respectively). It suggested that TSBM resulted in a less acidogenic protein source than SBM for dairy cows in early lactation receiving a concentrated diet. Hristov *et al.* [33] did not observe such result with late-lactation dairy cows fed diets composed with SBM and SoyPass® and characterized by different RDP level (9.4% and 11.6% on a DM basis) but not inducing ruminal acidosis (pH = 6.43 on average). Gressley and Armentano [34] tested two diets presenting similar RDP contents (10.1 vs. 7.4% on a DM basis) than those used in the present study and both inducing the same ruminal pH. However, their diets were not acidogenic (pH averaged 6.75) and moreover, the additional RDP for the high-RDP diet was supplied by urea whereas it dealed with a peptide and protein RDP source in the present study.

In our trial, decreasing the dietary RDP content, significantly reduced rumen N-NH_3 by 56%. Gressley and Armentano [34] observed such a decrease (−60%) with low RDP-diets (−27% relative to recommendation). Inadequate RDP, which cause reduced ruminal N-NH_3 concentrations, can lead to a depression in DMI. Our results showed that DMI was not significantly affected by the RDP level. These observations agreed with the results of Gressley and Armentano [34] who reported no change in DMI when a 7.4% RDP diet was fed as compared with a 10.1% RDP diet. Similarly, Kalscheur *et al.* [35] and, Reynal and Broderick [36] did not observe changes in DMI when RDP concentrations as low as 6.8% were fed. The LL diet provided NH_3-N content (35.5 mg/L) below the requirements of 61 mg/L found by Satter and Slyter [37] while ruminal NH_3-N for the AL diet was above (81.2 mg/L). Later, Hoover [38], indicated that the minimum rumen NH_3-N required for microbial CP production may be closer to 24 - 40 mg/L. As a result, LL seemed to induce a lack of soluble N in the rumen certainly propitious to decrease ruminal proteosynthesis as stated by Russell and Hino [39]. The tendency of a lower total PD urinary concentration (**Table 5**) and specifically of uric acid concentration corroborated this hypothesis. As a matter of fact, Johnson *et al.* [40] stated a positive relationship between uric acid urinary excretion and microbial N flow to the duodenum. Therefore, with the LL diet, ruminal NH_3-N was just adequate to support rumen microbial CP production but not sufficient to support ruminal digestion, particularly carbohydrate

digestion. This was supported by the observed concomittent decrease in OM rumen degradation. Part of the decrease of OM rumen degradation observed with LL could originate from less N rumen degradation (36.0% for LL vs. 56.2% for AL) on the one hand and, probably a lower degradation of carbohydrates on the other hand [41], as evidenced by decreased in total VFA and C3 concentrations (**Table 3**).

The use of TSBM in the diet resulted in a rise of E_h in the range of 19 mV compared to AL. The ruminal environment of dairy cows fed LL, *i.e.* fed a diet deficient in soluble N, was characterized by a reducing power about 10 times lower, as reflected by the observed rH values: 7.48 for LL vs. 6.36 for AL. As a result, the level of RDP, selected as a discriminating factor of the two experimental diets, lower for LL (7.5% DM) than for AL (10.6% DM), fed the cows would have an impact on the reducing power of the rumen. These physico-chemical observations were to be compared with the microbiological data obtained for both diets: the molecular fingerprint of bacterial communities differed significantly by 29% between cows receiving LL and AL. Thus, present results put forward that the ruminal reducing status varies with the diet of animals as already observed by Monteils *et al.* [42] and suggested by Julien *et al.* [16] in dairy cows fed a fiber- or a starch-based diet.

Regarding ruminal bacterial community, the lack of RDP seemed to penalize heavily the number of taxonomic units of the bacterial community (**Table 6**). Although it is important to keep in mind that at this level of number of peaks detected in this study, co-migration of 16S-stranded DNA extracted from bacterial communities could not be excluded [43], biasing the assessment of richness. Richness of the profiles was significantly correlated with rH confirming the possible relationship between the ruminal reducing level and the ruminal bacterial communities. The correlation (**Table 7**) between the richness and NH_3-N content highlighted the potential impact of the lack of soluble N on ruminal bacterial communities. Similarly, the diversity index-log Simpson, taking into account both the number of taxonomic units detected and their relative abundance was lower for LL than for AL (5.99 and 6.67, respectively). In addition, the microbial community structure observed for AL and LL was correlated with ruminal fermentative parameters, particularly with C2:C3 ratio induced by the diet and C3 content, as already observed by Michelland *et al.* [25] in dry-dairy cows. Those observations obviously highlighted the relationship between ruminal bacterial communities and fermentative activity.

Live yeast had significant effect on neither ruminal pH nor lactic acid content of the rumen, contrary to the observations of Marden *et al.* [3] in cows suffering from SARA with an initially high ruminal acid lactic content. In our study, lactic acid concentrations found 1 to 2 hours after feeding, *i.e.* at the peack of production [6] were much lower than those recorded by Marden *et al.* [3]. Nonetheless, they were consistent with those measured in cows receiving a diet composed by corn silage, hay and concentrates by Guedes *et al.* [44] who did not report ruminal pH values below 6.0 over an 8 h-post-feeding period. As a result, LY mode of action proposed by Marden *et al.* [3] was not verified in the present study, certainly because of less acidogenic ruminal conditions induced by both experimental diets.

However, the main results of the present study showed that LY supplementation increase OM and N total tract digestibility. Indeed, LY effect was more pronounced for LL diet: OM and N total tract digestibility increased by +3.7 and +5.9 points, respectively. It therefore suggested that differing LY effects on diet digestion depend on the dietary RDP content in early lactating dairy cows.

With LL, LY induced a higher ruminal total VFA content (+8.6%) which led to a numerical decrease of ruminal pH (−0.12 pH units). Live yeast seemed particularly to stimulate the production of C2 and C4 at the expense of C3 which would be a sign of an increasing activity of fibrolytic bacteria as already stated by Marden *et al.* [3]. In fact, these fermentative end-products are known to be produced by fibrolytic bacteria such as *Butyrivibrio fibrisolvens* whose activity could be promoted in the presence of LY as suggested by Julien *et al.* [45]. As a matter of fact, no effect of LY on structure, richness and diversity of ruminal bacterial communities was detected by means of CE-SSCP molecular fingerprints technique. However, using terminal restriction fragment length polymorphism technique which permit to define more precisely the composition of ruminal community and focus on some specific taxa, Pinloche *et al.* [46] showed that supplementation of the diet with LY in lactating dairy cows induced indeed an increase in the relative abondance of the taxa representing fibrolytic bacteria (*Fibrobacter*, *Ruminococcus* and *Eubacterium*) in the rumen. Meanwhile, these same authors also reported a decrease in the relative proportion of *Proteobacteria*, this bacteria being involved in ruminal proteolysis. The decrease in the proportion and/or activity of proteolytic bacteria in presence of LY could explain the reduction (5.2 points) of dietary N ruminal degradation observed with LL (**Table 4**).

The amount of N escaping from microbial degradation, estimated by calculation, was not altered by LY supplementation but the post-ruminal disparition of CP was increased: +3.5 points and +7.6 points for AL and LL,

respectively. This suggested that LY supplementation led to a shift in digestion from rumen to the intestine, specifically when ruminal CP degradation was limited. Indeed, few authors have focused on potential effects of LY on the part of post-rumen digestive tract of ruminant. However, in cows fed diet supplemented with LY, colonies of yeasts were counted in ileal and fecal contents [47] confirming the hypothesis of a potential probiotic activity in the large intestine.

5. Conclusion

The present study put forward that RDP content of diet fed lactating dairy cows could have a direct impact on the acidogenic capacity of the diet: TSBM resulted in less acidogenic than SBM used as main protein source in diet fed early-lactating dairy cows. The positive effect of LY on ruminal pH in cows receiving highly acidogenic diet and therefore suffering from subacute ruminal acidosis has already been demonstrated. The present study put forward that in the case of early-lactating dairy cows receiving diet with inadequate RDP content, LY revealed capable of modulating dietary N digestion. In fact, LY seemed to have a post-ruminal effect on N digestibility even more pronounced than the quantity of bypass N, *i.e.* with sources of protected dietary protein. In both cases, LY used as dietary feed additive permit a better utilization of diet in dairy cows.

Acknowledgements

The authors gratefully acknowledge the staff at the experimental station and particularly Brigitte Gestes, for their technical assistance.

References

[1] Jones, D.F., Hoover, W.H. and Miller Webster, T.K. (1998) Effects of Concentrations of Peptides on Microbial Metabolism in Continuous Culture. *Journal of Animal Science*, **76**, 611-616.

[2] Campanile, K.A., Zicarelli, F., Vecchio, D., Pacelli, C., Neglia, G., Balestrieri, A., Di Palo, R. and Infascelli, F. (2008) Effects of *Saccharomyces cerevisiae* on *in Vivo* Organic Matter Digestibility and Milk Yield in Buffalo Cows. *Livestock Science*, **114**, 358-361. http://dx.doi.org/10.1016/j.livsci.2007.11.002

[3] Marden, J.P., Julien, C., Monteils, V., Auclair, E., Moncoulon, R. and Bayourthe, C. (2008) How Does Live Yeast Differ from Sodium Bicarbonate to Stabilize Ruminal pH in High Yielding Dairy Cows? *Journal of Dairy Science*, **91**, 3528-3535. http://dx.doi.org/10.3168/jds.2007-0889

[4] Wang, C., Liu, Q., Yang, W.Z., Dong, Q., Yang, X.M., He, D.C., Zhang, P., Dong, K.H. and Huang, Y.X. (2009) Effects of Selenium Yeast on Rumen Fermentation, Lactation Performance and Feed Digestibilities in Lactating Dairy cows. *Livestock Science*, **126**, 239-244. http://dx.doi.org/10.1016/j.livsci.2009.07.005

[5] Chademana, I. and Offer, N.W. (1990) The Effect of Dietary Inclusion of Yeast Culture on Digestion in the Sheep. *Animal Production*, **50**, 483-489. http://dx.doi.org/10.1017/S0003356100004967

[6] Williams, P.E.V., Tait, C.A.G., Innes, G.M. and Newbold, C.J. (1991) Effects of the Inclusion of Yeast Culture (*Saccharomyces cerevisiae* plus Growth Medium) in the Diet of Dairy Cows on Milk Yield and Forage Degradation and Fermentation Patterns in the Rumen of Steers. *Journal of Animal Science*, **69**, 3016-3026.

[7] Moloney, A.P. and Drennan, M.J. (1994) The Influence of the Basal Diet on the Effects of Yeast Culture on Ruminal Fermentation and Digestibility in Steers. *Animal Feed Science and Technology*, **50**, 55-73. http://dx.doi.org/10.1016/0377-8401(94)90009-4

[8] Miranda, R.L.A., Mendoza, G., Barcena-Gama, R., Gonzalez, M.S.S., Ferrara, R., Ortega, M.E. and Cobos, P.M.A. (1996) Effect of *Saccharomyces cerevisiae* or *Aspergillus oryzae* Cultures and NDF Level on Parameters of Ruminal Fermentation. *Animal Feed Science and Technology*, **63**, 289-296. http://dx.doi.org/10.1016/S0377-8401(96)01008-5

[9] Lascano, G.J. and Heinrichs, A.J. (2009) Rumen Fermentation Pattern of Dairy Heifers Fed Restricted Amounts of Low, Medium, and High Concentrate Diets without and with Yeast Culture. *Livestock Science*, **124**, 48-57. http://dx.doi.org/10.1016/j.livsci.2008.12.007

[10] Lascano, G.J., Zanton, G.I. and Heinrichs, A.J. (2009) Concentrate Levels and *Saccharomyces cerevisiae* Affect Rumen Fluid-Associated Bacteria Numbers in Dairy Heifers. *Livestock Science*, **126**, 189-194. http://dx.doi.org/10.1016/j.livsci.2009.06.019

[11] Chaucheyras-Durand, F., Walker, N.D. and Bach, A. (2008) Effects of Active Dry Yeasts on the Rumen Microbial Ecosystem: Past, Present and Future. *Animal Feed Science and Technology*, **145**, 5-26. http://dx.doi.org/10.1016/j.anifeedsci.2007.04.019

[12] Sniffen, C.J., Chaucheyras-Durand, F., De Ondarza, M.B. and Donaldson, G. (2004) Predicting the Impact of a Live Yeast Strain on Rumen Kinetics and Ration Formulation. *Proceedings of the* 19*th Annual Southwest Nutrition and Management Conference*, Tempe, 24-25 February 2004, 53-59.

[13] Giger-Reverdin, S., Bezault, N., Sauvant, D. and Bertin, G. (1996) Effects of a Probiotic Yeast in Lactating Ruminants: Interaction with Dietary Nitrogen Level. *Animal Feed Science and Technology*, **63**, 149-162. http://dx.doi.org/10.1016/S0377-8401(96)01011-5

[14] Streeter, M.N., Wagner, D.G., Hibberd, C.A. and Owens, F.N. (1990) Comparison of Corn with Four Sorghom Grain Hybrids: Site and Extent of Digestion in Steers. *Journal of Animal Science*, **68**, 3429-3440.

[15] NRC (2001) Nutrient Requirements of Dairy Cattle. 7th Revised Edition, National Academy of Sciences, Washington DC.

[16] Julien, C., Marden, J.P., Bonnefont, C., Moncoulon, R., Auclair, E., Monteils, V. and Bayourthe, C. (2010) Effects of Varying Proportions of Concentrates on Ruminal-Reducing Power and Bacterial Community Structure in Dry Dairy Cows Fed Hay-Based Diets. *Animal*, **4**, 1641-1646. http://dx.doi.org/10.1017/S1751731110000972

[17] Orskov, E.R. and McDonald, I. (1979) Estimation of Protein Degradability in the Rumen from Incubation Measurements Weighted According to Rate of Passage. *Journal of Agricultural Science*, **92**, 499-503. http://dx.doi.org/10.1017/S0021859600063048

[18] Playne, M.J. (1985) Determination of Ethanol, Volatile Fatty Acids, Lactic Acid and Succinic Acid in Fermentation Liquids by Gas Chromatography. *Journal of the Science of Food and Agriculture*, **36**, 638-644. http://dx.doi.org/10.1002/jsfa.2740360803

[19] Verdouv, H., Van Echteld, C.J.A. and Dekkers, E.M.J. (1978) Ammonia Determination Based on Indophenol Formation with Sodium Salicylate. *Water Research*, **12**, 399-402. http://dx.doi.org/10.1016/0043-1354(78)90107-0

[20] Krom, M.D. (1980) Spectrophotometric Determination of Ammonia: A Study of a Modified Berthelot Reaction Using Salicylate and Dichloroisocyanurate. *Analyst*, **105**, 305-316. http://dx.doi.org/10.1039/an9800500305

[21] Houba, V.J.G., Novozamsky, I., Uittenbogaard, J. and Van Der Lee, J.J. (1987) Automatic Determination of Total Soluble Nitrogen in Soil Extracts. *Landwirtschaftliche Forschung*, **40**, 295-302.

[22] George, S.K., Dipu, M.T., Mehra, U.R., Singh, P., Verma, A.K. and Ramgaokar, J.S. (2006) Improved HPLC Method for the Simultaneous Determination of Allantoin, Uric Acid and Creatinine in Cattle Urine. *Journal of Chromatography B*, **832**, 134-137. http://dx.doi.org/10.1016/j.jchromb.2005.10.051

[23] Delbès, C., Godon, J.J. and Moletta, R. (1998) 16S rDNA Sequence Diversity of a Culture-Accessible Part of an Anaerobic Digestor Bacterial Community. *Anaerobe*, **4**, 267-275. http://dx.doi.org/10.1006/anae.1998.0176

[24] Zumstein, E., Moletta, R. and Godon, J.J. (2000) Examination of Two Years of Community Dynamics in an Anaerobic Bioreactor Using Fluorescence Polymerase Chain Reaction (PCR) Single-Strand Conformation Polymorphism Analysis. *Environmental Microbiology*, **2**, 69-78. http://dx.doi.org/10.1046/j.1462-2920.2000.00072.x

[25] Michelland, R.J., Monteils, V., Zened, A., Combes, S., Cauquil, L., Gidenne, T., Hamelin, J. and Fortun-Lamothe, L. (2009) Spatial and Temporal Variations of the Bacterial Community in the Bovine Digestive Tract. *Journal of Applied Microbiology*, **107**, 1642-1656. http://dx.doi.org/10.1111/j.1365-2672.2009.04346.x

[26] Michelland, R.J., Dejean, S., Combes, S., Fortun-Lamothe, L. and Cauquil, L. (2009) StatFingerprints: A Friendly Graphical Interface Program for Processing and Analysis of Microbial Fingerprint Profiles. *Molecular Ecology Resources*, **9**, 1359-1363. http://dx.doi.org/10.1111/j.1755-0998.2009.02609.x

[27] R Development Core Team (2009) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna. http://www.R-project.org

[28] Marounek, M., Roubal, P. and Bartos, S. (1987) The Redox Potential, rH and pH Values in the Gastrointestinal Tract of Small Ruminants. *Physiologia Bohemoslovaca*, **36**, 71-74.

[29] Valadares, R.F.D., Broderick, G.A., Valadares Filho, S.C. and Clayton, M.K. (1999) Effect of Replacing Alfalfa Silage with High Moisture Corn on Ruminal Protein Synthesis Estimated from Excretion of Total Purine Derivatives. *Journal of Dairy Science*, **82**, 2686-2696. http://dx.doi.org/10.3168/jds.S0022-0302(99)75525-6

[30] Sauvant, D., Meschy, F. and Mertens, D.R. (1999) Les composantes de l'acidose ruminale et les effets acidogènes des rations. *INRA Productions Animales*, **12**, 49-60.

[31] Sauvant, D., Giger-Reverdin, S. and Meschy, F. (2006) Le contrôle de l'acidose ruminale latente. *INRA Productions Animales*, **19**, 69-78.

[32] Lechartier, C. and Peyraud, J.L. (2010) The Effects of Forage Proportion and Rapidly Degradable Dry Matter from Concentrate on Ruminal Digestion in Dairy Cows Fed Corn Silage-Based Diets with Fixed Neutral Detergent Fiber and Starch Contents. *Journal of Dairy Science*, **93**, 666-681. http://dx.doi.org/10.3168/jds.2009-2349

[33] Hristov, A.N., Etter, R.P., Ropp, J.K. and Grandeen, K.L. (2004) Effect of Dietary Crude Protein Level and Degrad-

ability on Ruminal Fermentation and Nitrogen Utilization in Lactating Dairy Cows. *Journal of Animal Science*, **82**, 3219-3229.

[34] Gressley, T.F. and Armentano, L.E. (2007) Effects of Low Rumen-Degradable Protein or Abomasal Fructan Infusion on Diet Digestibility and Urinary Nitrogen Excretion in Lactating Dairy Cows. *Journal of Dairy Science*, **90**, 1340-1353. http://dx.doi.org/10.3168/jds.S0022-0302(07)71621-1

[35] Kalscheur, K.F., Baldwin, R.L., Glenn, B.P. and Kohn, R.A. (2006) Milk Production of Dairy Cows Fed Differing Concentrations of Rumen-Degraded Protein. *Journal of Dairy Science*, **89**, 249-259. http://dx.doi.org/10.3168/jds.S0022-0302(06)72089-6

[36] Reynal, S.M. and Broderick, G.A. (2005) Effect of Dietary Level of Rumen-Degraded Protein on Production and Nitrogen Metabolism in Lactating Dairy Cows. *Journal of Dairy Science*, **88**, 4045-4064. http://dx.doi.org/10.3168/jds.S0022-0302(05)73090-3

[37] Satter, L.D. and Slyter, L.L. (1974) Effect of Ammonia Concentration on Rumen Microbial Protein Production *in Vitro*. *British Journal of Nutrition*, **22**, 199-208. http://dx.doi.org/10.1079/BJN19740073

[38] Hoover, W.H. (1986) Chemical Factors Involved in Ruminal Fiber Digestion. *Journal of Dairy Science*, **69**, 2755-2766. http://dx.doi.org/10.3168/jds.S0022-0302(86)80724-X

[39] Russell, J.B. and Hino, T. (1985) Regulation of Lactate Production in *Streptococcus bovis*: A Spiraling Effect That Contributes to Rumen Acidosis. *Journal of Dairy Science*, **68**, 1712-1721. http://dx.doi.org/10.3168/jds.S0022-0302(85)81017-1

[40] Johnson, L.M., Harrison, J.H. and Riley, R.E. (1998) Estimation of the Flow of Microbial Nitrogen to the Duodenum Using Urinary Uric or Allantoin. *Journal of Dairy Science*, **81**, 2408-2420. http://dx.doi.org/10.3168/jds.S0022-0302(98)70132-8

[41] Peyraud, J.L., Le Liboux, S. and Vérité, R. (1997) Effet du niveau et de la nature de l'azote dégradable sur la digestion ruminale d'un régime à base d'ensilage de maïs chez la vache laitière. *Reproduction Nutrition Development*, **37**, 313-328. http://dx.doi.org/10.1051/rnd:19970307

[42] Monteils, V., Rey, M. and Gidenne, T. (2009) Mid to Long Term Stability of Ruminal Physicochemistry in Dairy Cows Fed a Fibre- or a Starch-Based Diet. *Proceedings of the* 11*th International Symposium on Ruminant Physiology*, Clermont-Ferrand, 6-9 September 2009, 284-285.

[43] Loisel, P., Harmand, J., Zemb, O., Latrille, E., Lobry, C., Delgenes, J.P. and Godon, J.J. (2006) Denaturing Gradient Electrophoresis (DGE) and Single-Strand Conformation Polymorphism (SSCP) Molecular Fingerprintings Revisited by Simulation and Used as a Tool to Measure Microbial Diversity. *Environmental Microbiology*, **8**, 720-731. http://dx.doi.org/10.1111/j.1462-2920.2005.00950.x

[44] Guedes, C.M., Gonçalves, D., Rodrigues, M.A.M. and Dias-da-Silva, A. (2008) Effects of a *Saccharomyces cerevisiae* Yeast on Ruminal Fermentation and Fibre Degradation of Maize Silages in Cows. *Animal Feed Science and Technolohy*, **145**, 27-40. http://dx.doi.org/10.1016/j.anifeedsci.2007.06.037

[45] Julien, C., Marden, J.P., Enjalbert, F., Bayourthe, C. and Troegeler-Meynadier, A. (2010) Live Yeast as a Possible Modulator of Polyunsaturated Fatty Acid Biohydrogenation in the Rumen. *Revue Médecine Vétérinaire*, **161**, 391-400.

[46] Pinloche, E., McEvan, N., Marden, J.P., Bayourthe, C., Moncoulon, R. and Newbold, C.J. (2008) Effect of Yeast on the Bacterial Diversity of the Rumen. *Proceedings of the* 6*th INRA-RRI Symposium of Gut Microbiology*, Clermont-Ferrand, 18-20 June 2008, 9.

[47] Monteils, V., Cauquil, L., Auclair, E. and Bayourthe, C. (2006) Behaviour of Live Yeast BIOSAF Sc 47 during Digestive Transit in Dairy Cows. *Proceedings of the* 4*th INRA-RRI Symposium of Gut Microbiology*, Aberdeen, 21-23 June 2006, S106-S107.

Scientific Research Publishing (SCIRP) is one of the largest Open Access journal publishers. It is currently publishing more than 200 open access, online, peer-reviewed journals covering a wide range of academic disciplines. SCIRP serves the worldwide academic communities and contributes to the progress and application of science with its publication.

Other selected journals from SCIRP are listed as below. Submit your manuscript to us via either submit@scirp.org or Online Submission Portal.