Live yeast as a possible modulator of poly-unsaturated fatty acid biohydrogenation in the rumen

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SUMMARY

In dairy cows, several studies focused on the effects of sodium bicarbonate and fibre on ruminal linoleic acid (*c*9*c*12-C18:2) biohydrogenation (BH) whereas literature is scarce about the effect of live yeast, used as a feed additive. The objective of this *in vivo* study was to evaluate the capacity of two dietary feed additives, sodium bicarbonate and live yeast (Strain Sc47), and hay to modulate ruminal BH and particularly conjugated linoleic acids (CLA) and *trans*-monoenoic acids (*t*-C18:1) production. Four dry dairy cows fitted with ruminal cannula, were used in a 4×4 Latin square design. They were given a control diet (CD) at a daily feeding rate of 10.4 kg of dry matter/cow supplemented with 100 g/d of sodium bicarbonate or 5 g/d of live yeast or a hay diet formulated to provide the same main fatty acids (FA) as CD during a 14-d experimental period. Ruminal pH and redox potential were measured from 1 h before feeding to 8 h after, and ruminal fluid samples were taken at 5 h after feeding for volatile fatty acids, ammonia and fatty acid determination. In addition to the *in vivo* experiment, an *in vitro* experiment was carried out to ascertain the possible mode of action of live yeast on *c*9*c*12-C18:2 BH: ruminal fluid was obtained from a donor cow fed with hay and was incubated in batch cultures over 6 h with a 6-pH buffer using starch, urea and grape seed oil as substrates. Results gathered from both experiments suggested that live yeast supplement increased the accumulation of *t*-C18:1 compared to sodium bicarbonate and prevented the formation of C18:0 which is usually observed when hay is added to a high concentrate diet. The accumulation of *t*-C18:1 observed in presence of live yeast was probably due to an inhibition of the second reduction step as a result of a more complete isomerisation of *c*9*c*12-C18:2.

Keywords: Cow, ruminal biohydrogenation, live yeast, fatty acids, conjugated linoleic acid.

RÉSUMÉ

La levure vivante, un modulateur potentiel de la biohydrogénation ruminale des acides gras polyinsaturés

Chez les vaches laitières, plusieurs études montrent les effets du bicarbonate de sodium et des fibres sur la biohydrogénation (BH) de l'acide linoléique (*c*9*c*12-C18 :2) dans le rumen alors qu'elles sont rares en ce qui concerne ceux de la levure vivante, utilisée comme additif dans l'alimentation. L'objectif de cette étude conduite *in vivo* était d'évaluer les capacités de deux additifs alimentaires, le bicarbonate de sodium et la levure vivante (souche Sc 47), ainsi que du foin à moduler la BH ruminale et en particulier la production d'acides linoléiques conjugués (CLA) et d'acides trans-monoenoiques (*t*-C18:1). Quatre vaches laitières taries, munies d'une canule ruminale, ont été utilisées dans un carré latin 4 × 4. Elles ont reçu individuellement environ 10,4 kg de matière sèche (MS)/j d'un régime témoin (CD) additionné de 100 g/j de bicarbonate de sodium ou de 5 g/j de levures vivantes, ou un régime alimentaire contenant du foin grossier formulé pour offrir des teneurs en acides gras (AG) identiques à celles fournies par CD, pendant une période expérimentale de 14 jours. Le pH et le potentiel rédox du rumen ont été mesurés à partir de 1 h avant le repas jusqu'à 8 h après, et des échantillons de contenu ruminal liquide ont été prélevés 5 h après le repas pour le dosage des concentrations en acides gras volatiles, en ammoniac et des teneurs en acides gras. En outre, en parallèle de l'expérience *in vivo*, une expérience *in vitro* a été réalisée pour préciser les voies d'action des levures vivantes sur la BH du *c*9*c*12-C18:2 sous conditions contrôlées: le contenu ruminal a été prélevé sur des vaches donneuses nourries au foin et incubé pendant 6 h en milieu tamponné à pH 6 en utilisant comme substrats de l'amidon, de l'urée et de l'huile de pépins de raisin. L'analyse combinée des résultats des deux expériences suggère que les levures vivantes ont induit une accumulation plus importante de *t*-C18:1 que le bicarbonate de sodium et qu'elles ont freiné la formation de C18:0 habituellement observée lors de l'ajout de foin dans un régime riche en concentrés. L'accumulation de *t*-C18:1 en présence de levures vivantes serait probablement due à l'inhibition de la seconde réduction induite par une meilleure efficacité de l'isomérisation de *c*9*c*12-C18:2.

Mots clés : Vache, biohydrogénation ruminale, levure vivante, acides gras, acide linoléique conjugué.

Introduction

Conjugated linoleic acids (CLA) constitute a group of positional and geometric isomers of linoleic acid (*c*9*c*12-C18:2) with conjugated double bonds. These fatty acids (FA) have been reported to possess some potent effects on human health: *c*9*t*11-CLA is known to prevent some diseases like cancer, obesity and atherosclerosis in animal models [23] whereas *t*10*c*12-CLA has been shown to exhibit some detrimental effects on human health, for example it favours tumorogenesis and cardiovascular diseases [11, 31, 39]. In human diets, the richest sources of CLA are ruminant dairy products [32]. Milk CLA isomers are produced during the ruminal biohydrogenation (BH) of *c*9*c*12-C18:2, and most milk *c*9*t*11-CLA results from the mammary desaturation of vaccenic acid (*t*11-C18:1) [9], another BH intermediate.

According to TURPEINEN *et al*. [36], this desaturation also occurs in human tissues, so that an increase in *t*11 isomers in milk should lead to *c*9*t*11-CLA production and be favourable for humans. These considerations underline the necessity to control ruminal BH of unsaturated FA. Ruminal BH corresponds to the microbial reduction of unsaturated FA. For example (Figure 1), BH of *c*9*c*12-C18:2 is divided into three steps [33]: first, an isomerisation into CLA, then a first reduction producing *trans*-monoenoic acids (*t*-C18:1), and a final second reduction producing stearic acid (C18:0). The first reaction is catalyzed by various enzymes synthesised by different bacteria, leading to several CLA isomers. The predominant biohydrogenating bacterium, *Butyrivibrio fibrisolvens*, a fibrolytic one, isomerises *c*9*c*12-C18:2 into c9t11-CLA and *t*9*t*11-CLA, and hydrogenates them mainly to *t*11-C18:1; *Butyrivibrio proteoclasticus* (formerly *Clostridium proteoclasticum* [21]) is also able to perform these reactions [18]. Isomerisation of *c*9*c*12-C18:2 into *t*10*c*12-CLA would be due to lactate consuming bacteria *Megasphera elsdenii* and/or *Propionibacterium acnes* [14, 38]. However, this is debatable, and the bacterium able to hydrogenate this CLA isomer into *t*10-C18:1 or *c*12-C18:1 is unknown. Bacteria able to perform the last of *c*9*c*12-C18:2 BH reactions are rarely studied and therefore unknown.

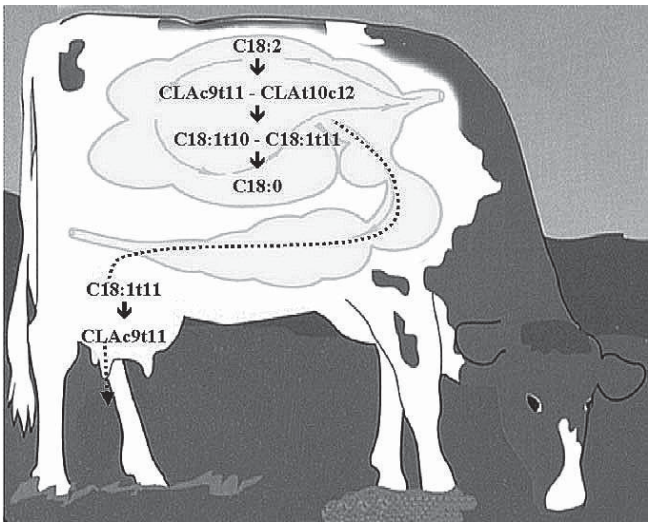

FIGURE 1: Ruminal biohydrogenation of linoleic acid and mammary desaturation of vaccenic acid (with copyright)..

Addition of fibre in diets results in an increased efficiency of ruminal BH [6] that can be overcome by the addition of sodium bicarbonate (SB) [13]. On the contrary, no literature data are available on the effects of live yeast on BH. Live yeast used as a ruminant dietary feed additive is known to impact the ruminal microflora involved in BH. Indeed, live yeast improved growth and activity of ruminal lactate-consuming bacteria, like *M. elsdenii* [27] or *S. ruminantium* [22, 25], Actinobacteria, including *P. acnes* [25] as well as fibrolytic bacteria [5, 17]. Consequently, live yeast could act at different steps of BH namely, firstly by modulating biohydrogenating microorganisms, i.e. promoting growth of either *t*11 or *t*10 isomer producing bacteria and secondly by modulating the ruminal biotope, i.e. by stabilising ruminal pH or favouring stronger reducing conditions [20].

Since the influence of live yeast on the ruminal biotope is clearer with high concentrate diets [8], the objective of this study was to investigate *in vivo* the effects of live yeast on ruminal BH in cows fed with a high concentrate diet, and to compare the effects of live yeast with those of well known modulators of BH: SB and fibre [6, 35]. Thus, to go a step further, an *in vitro* study was carried out at pH 6, during 6-h incubation, with starch as the main fermentative substrate in order to focus on the possible live yeast effect on ruminal BH and specifically on fibrolytic bacteria which are known to be in part involved in ruminal BH reactions and *t*11 isomer production.

Materials and Methods

EXPERIMENTAL DESIGNS

In vivo experiment (experiment 1)

Four ruminally cannulated non-lactating Holstein cows were used in a 4 × 4 Latin square design. Cannulation techniques [30] allowed for humane treatment of cows and adhered to locally approved procedures. Animals were housed in individual tie stalls throughout the experiment with free access to water. They were assigned to one of 4 treatments: a control diet (CD) based on maize silage, CD plus 100 g/d of sodium bicarbonate (SBD), CD plus 5 g/d of live *Saccharomyces cerevisiae* (10^{10} CFU/g DM, ACTISAF® Sc 47, Lesaffre Feed Additives, France) (YD) and a hay diet (HD) in which a part of maize silage was replaced by hay (71% of Neutral Detergent Fibre (NDF) and 42% of Acid Detergent Fibre (ADF) on the dry matter (DM) basis), with the same quantity of FA than CD (Table I). During each 14-d experimental period (10 d of adaptation to the diet and 4 d of measurement) the daily feeding rate was adjusted to 10.4 kg of DM/cow on average, in order to avoid sorting and orts. The diets were offered as a total mixed ration twice daily in equal portions at 09.00 and 17.00 h. Live yeast and sodium bicarbonate doses were top-dressed on the rations at each meal.

During the first three days of measurement, for each cow, ruminal pH and redox potential (E_h) were recorded hourly over a 9 hours period from 1 hour before to 8 hours after the morning meal (T-1 to T+8), according to a method adapted from MARDEN *et al*. [19]. Since an Ag-AgCl reference electrode was used (Metrohm, Herisau, Switzerland), all measured values were corrected using the formula: $E_h = E_0 + C$, where E_0 is the potential of the platinum electrode and C is the potential of the Ag-AgCl reference electrode compared with the Standard Hydrogen Electrode, *i.e.* + 199 mV at 39°C. A 50-mL ruminal fluid sample was sucked out of the rumen of each cow at T_{+5}. One part (10-mL) was preserved by the addition of 1 mL of mercuric chloride (2% wt/v) for subsequent volatile FA (VFA) and ammonia N (NH_3-N) determination. The other part (40-mL) of sampled ruminal fluid was used for subsequent FA determination. All the samples were kept at –18 °C until their respective analysis.

In vitro experiment (experiment 2)

Ruminal fluid was obtained from two fistulated dry dairy cows receiving a fixed quantity of alfalfa hay (5.6 kg

Item	Control diet (CD)	Hay diet (HD)
Ingredient (% of DM)		
Maize silage	38.6	15.7
Soybean meal	6.8	5.8
Ground maize	34.8	42.1
Hay	-	17.0
Extruded soybean	17.9	17.5
Mineral vitamin premix[1]	1.9	1.9
Nutrient analysis		
DM (% of raw matter)	53.5	70.3
NDF (% DM)	25.7	30.8
ADF (% DM)	12.2	15.6
Crude protein (%DM)	17.1	16.8
Starch (%DM)	33.8	30.8
FA composition (%DM)		
Total fatty acids	5.0	4.7
C16:0	0.6	0.6
C18:0	0.2	0.2
*c*9-C18:1	1.0	0.9
*c*9*c*12C18:2	2.6	2.5
*c*9*c*12*c*15C18:3	0.4	0.4

DM: Dry matter; [1]Contained (per kg of premix, DM basis) P: 40g, Ca: 260g, Mg: 50g, Na: 20g, Zn: 5g, Mn: 4g, Cu: 1g, I: 40 mg, Co: 20 mg, Se: 20mg, vitamin A: 450 000 IU, vitamin D_3: 100 000 IU and vitamin E: 1 500 IU; NDF: Neutral Detergent Fibre; ADF: Acid Detergent Fibre; FA: fatty acids.

TABLE I: Composition of the control diet (CD) and hay diet (HD) on a dry matter (DM) basis (%).

DM/day) plus minerals and meadow hay *ad libitum*, in order to enhance fibrolytic bacterial populations. On two different days (*i.e*. two series of incubation), ruminal fluid was taken from each cow with a vacuum pump 30 min after feeding, and strained through a metal sieve (1.6 mm mesh). Strained ruminal fluid was then transferred to the laboratory under anaerobic conditions at 39°C, gassed with CO_2 and centrifuged (150g, 5 min, 39°C) in order to remove fibre particles and to obtain an inoculum containing less than 1% of NDF and ADF on DM basis. The supernatant (80 mL) and a pH 6 buffer solution (80 mL; 64.09 mM KH_2PO_4, 1.03 mM Na_2HPO_4, 27.50 mM Na_2HCO_3, 12.06 mM NaCl, 9.05 mM KCl, 0.52 mM $CaCl_2$ and 0.36 mM $MgSO_4$) prewarmed at 39°C and saturated with CO_2, were poured into a 250-mL Erlenmeyer flask containing 1.5 g of corn starch (purity 99%, Sigma-Aldrich), 0.15 g of urea (purity 99.5%, Prolabo) and 200 mg of grape seed oil (67.2% of *c*9,*c*12-C18:2, 20.2% of oleic acid, *c*9-C18:1, and 0.5% of linolenic acid, *c*9,*c*12,*c*15-C18:3 /total FA). Live *Saccharomyces cerevisiae* (10^{10} CFU/g DM, ACTISAF® Sc 47, Lesaffre Feed Additives, France) was added at a dose of 0.01g per flask in 12 of the 24 incubated flasks. Eight non-incubated flasks without added FA were also prepared, and immediately frozen at –18°C, in order to determine the initial quantities of FA (Table II). The other flasks were placed in a waterbath rotary shaker (Aquatron; Infors AG, 4103 Bottmingen, Germany) at 39°C after gassing with CO_2. They were closed by a cap equipped with a tube that extremity dived into water, in order to clear out fermentation gas without entrance of oxygen,, stirred at 130 rpm and kept in the dark throughout the incubation.

Fatty acids	Quantity[1] (mg/flask)
C18:0	9.40
*c*9-C18:1	27.69
*t*11-C18:1	0.89
*c*9,*c*12-C18:2	89.69
*c*9,*t*11-CLA2	1.00
*c*9,*c*12,*c*15-C18:3	0.99

[1]Quantity in non incubated flasks plus quantity provided by 200 mg of grape seed oil; 2: CLA: Conjugated Linoleic Acid.

TABLE II: Initial quantities of unsaturated C18 fatty acids (FA) including intermediates of linoleic acid biohydrogenation (CLA and *t*-C18:1) in the media of flasks before incubation.

After 6 hours incubation, the flasks were placed into iced water to stop any bacterial activity. The contents of the flasks were frozen at –18°C, freeze-dried (Virtis Freezemobile 25; Virtis, Gardiner, NY), weighed, ground and homogenised in a ball mill (Dangoumau, distributed by Prolabo, Nogent-sur-Marne, France), and kept at –18 °C for further analysis.

CHEMICAL ANALYSIS

Chemical compositions were determined by the official methods: NF V18-100 [1] for crude protein, NF V18-121 [2] for starch and NF V18-122 [3] for NDF. The concentrations

of VFA were determined using the gas chromatographic method of Playne [26], modified by MARDEN *et al*. [20]. The determination of NH_3-N was based on the modified Berthelot reaction with the Skalar Method [10, 16, 37].

The fatty acids were extracted and methylated *in situ* with the procedure of PARK and GOINS [24], except that the solution of 14% of borontrifluoride in methanol was replaced by a solution of methanol-acetyl chloride. The nonadecanoic acid (C10:1) was used as internal standard. The FA methyl esters were then quantified by GC [Agilent 6890N, equipped with a model 7683 auto injector (Network GC System, Palo Alto, California, USA) and with a fused silica capillary column (CPSil88, 100 m x .25 mm ID, 0.20 µm film thickness; Chrompack-Varian, Middleburg, Netherlands)]. Flame ionization detector temperature was maintained at 260°C and the injector at 255°C, the split ratio was 1:50. Hydrogen was the carrier gas with a constant flow of 1 mL/min. The samples were injected in 1 µL of hexane with an automatic injector. Initial temperature of oven was 60°C, held for 2 min, increased by 8°C/min to 150°C, held at 150°C for 12 min, increased by 2°C/min to 175°C, held at 175°C for 20 min, increased by 5°C/min to 225°C, maintained at 225°C for 10 min and increased by 5°C/min to a final temperature of 240°C. This method did not allow the separation of *t*13 and *t*14-C18:1 which coelute with *c*9-C18:1 and did not allow the separation of *c*9,*c*12,*c*15-C18:3 from C20:1. Consequently, a second method was performed to separate these coeluted FA. For this analysis, flame ionization detector temperature was maintained at 260°C and the injector at 255°C, the split ratio was 1:75. Hydrogen was the carrier gas with a constant flow of 1 mL/min. The samples were injected in 0.5 µl of hexane with an automatic injector. Initial temperature of oven was 60°C, held for 3 min, increased by 8°C/min to 190°C, held at 190°C for 13 min, increased by 5°C/min to 225°C, held at 225°C for 10 min, increased by 10°C/min to a final temperature of 230°C and maintained 1 min. Peaks were identified and quantified by comparison with commercial standards (Sigma, St. Louis, Missouri, USA), except *t*-C18:1 isomers other than *t*9 and *t*11 C18:1, which were identified by order of elution.

CALCULATIONS AND STATISTICAL ANALYSIS

All data were analysed using the General Linear Model (GLM) of SYSTAT (Version 9, SPSS Inc., 1998, Chicago, IL) and were reported as mean values with standard error of the mean (SEM). Differences were considered significant at $P < 0.05$ and trends were discussed at $P \leq 0.10$.

For the in vivo experiment (experiment 1), data for fermentative parameters and FA were analyzed with a GLM model including the effects of treatment, period and cow, and differences between treatment effects were assessed by pair wise comparisons (Tukey's Test). Responses of pH and E_h were analyzed using a repeated-measures model which included as main plot the effects of cow, treatment and period whereas sampling time and the interaction between treatment and sampling time were considered in the subplot.

Initial quantities of unsaturated C18 FA including intermediates of *c*9*c*12-C18:2 BH were calculated by addition of the quantity in non incubated flasks plus the quantity from 200 mg of grape seed oil during the *in vitro* experiment (experiment 2). The rates (mg/L/h) and the efficiencies given by the reaction speed (V_i) and the substrate loss ratio (R_i) of the three steps of *c*9*c*12-18:2 ruminal BH were calculated as described by TROEGELER-MEYNADIER *et al.* [34]. Briefly, the following formulas were used:

For the *c*9,*c*12-C18:2 isomerisation (reaction 1):
V_1 = ([C18:2]b – [C18:2]e) / 6,
E_1 = ([C18:2]b – [C18:2]e) / [C18:2]b,
where [C18:2]b and [C18:2]e were the C18:2 concentrations at the beginning and at the end of the 6 h incubation, respectively.

For the CLA reduction (reaction 2):
V_2 = ([C18:2]b – [C18:2]e +[CLA]b – [CLA]e) / 6,
E_2 = ([C18:2]b – [C18:2]e + [CLA]b – [CLA]e) / ([C18:2]b – [C18:2]e + [CLA]b),
where [CLA]b and [CLA]e were the concentrations of total CLA isomers measured at the beginning and at the end of the 6 h incubation, respectively.

For the trans-C18:1 reduction (reaction 3):
V_3 = ([C18:2]b – [C18:2]e +[CLA]b – [CLA]e + [*trans*-C18:1]b – [*trans*-C18:1]e) / 6,
E_3 = ([C18:2]b – [C18:2]e + [CLA]b – [CLA]e + [*trans*-C18:1]b – [*trans*-C18:1]e) / ([C18:2]b – [C18:2]e + [CLA]b – [CLA]e + [*trans*-C18:1]b),
where [trans-C18:1]b and [trans-C18:1]e were the concentrations of total trans-C18:1 isomers measured at the beginning and at the end of the 6 h incubation, respectively.

All data were analysed using an univariate GLM model including the effects of treatment and series of incubation.

Results

EXPERIMENT 1

Ruminal pH and E_h did not differ among treatments (Table III) averaging 6.37 and -203 mV, respectively. The ruminal concentration of total VFA measured 5 hours after the morning meal averaged 84.9 m*M* and did not differ among treatments. The ruminal propionate concentrations were significantly higher in the control diet (CD) than in the hay diet ($P = 0.010$). Butyrate and valerate concentrations in rumen were significantly increased when cows received the live yeast-added diet (YD) compared to cows fed with the hay diet (HD) ($P < 0.05$ and $P < 0.01$, respectively) whereas acetate, isobutyrate and isovalerate contents did not significantly differ according to the diets. The NH_3-N concentration measured at T_{+5} was significantly higher with HD than with the three others treatments ($P < 0.001$).

Ruminal FA profiles were clearly affected by treatment (Table IV). The percentages of C6:0 and of C7:0 at a lesser extend with YD were higher ($P < 0.01$ and $P < 0.10$ respectively) than with CD and SBD but remained similar to value observed with HD. The percentages of some saturated linear fatty acids (C18:0, C12:0, C13:0) were significantly lowered

Parameters	Diets					*P* value
	CD	SBD	YD	HD	SEM	
E_h (mV)	-202	-203	-201	-205	9.35	NS
pH	6.36	6.39	6.31	6.40	0.07	NS
Total VFA (mM)	87.07	83.31	86.12	84.39	1.20	NS
Acetate (mM)	51.77	50.01	51.61	54.44	0.78	NS
Propionate (mM)	21.06^{a}	19.14^{ab}	18.87^{ab}	17.00^{b}	0.40	0.010
Isobutyrate (mM)	1.13	1.17	1.20	1.12	0.02	NS
Butyrate (mM)	8.87^{ab}	9.64^{ab}	10.88^{a}	8.74^{b}	0.27	< 0.05
Isovalerate (mM)	2.04	2.08	2.19	1.99	0.05	NS
Valerate (mM)	1.19^{ab}	1.26^{ab}	1.37^{a}	1.10^{b}	0.03	< 0.01
NH_3-N (mg/L)	49.75^{b}	49.76^{b}	62.85^{b}	95.80^{a}	4.35	< 0.001

NS: Not significant; VFA: volatile fatty acids.
Different superscripts ^{a,b,} within a same row indicate significant differences (P < 0.05).

TABLE III: Physico-chemical and fermentative parameters, and carbohydrate degradation according to the dairy cow diets: CD (control diet), SBD (sodium bicarbonate diet), HD (hay diet) and YD (live yeast diet). Results are expressed as means associated to standard error of the mean (SEM).

with YD compared to the control diet (for C12:0 and C13:0, *P < 0.05*) or to the hay diet for C18:0 (*P < 0.05*). The same trend was also observed for C14:0. Furthermore, percentages of C15:0 and of some branched fatty acids (C13:0*iso*, C13:0*anteiso* and C15:0*anteiso*) as well as the percentage of the monounsaturated acid C17:1 were higher with HD than with other diets (*P < 0.05* to *P < 0.001*), while the percentage of C15:0*iso* with HD and CD was higher than with SBD and YD. The percentage of *c*9-C18:1 remained unchanged with treatment averaging 1.8%. The percentages of *c*12-C18:1 and *c*15-C18:1 were significantly increased with YD than with the other diets (*P < 0.001*). The percentages of *t*5-C18:1, *t*6+*t*7+*t*8-C18:1, *t*9-C18:1, *t*12-C18:1, *t*15-C18:1 and *t*16-C18:1 with YD were significantly higher than with the other diets (*P < 0.01* to *P < 0.001*). Percentages of *t*10-C18:1 and *t*11-C18:1 were also numerically higher with YD than with other diets but, no significant difference was found between YD and CD for *t*10-C18:1 (*P = 0.675*) and between YD and SBD for *t*11-C18:1 (*P = 0.174*). The percentage of *t*13 + *t*14-C18:1 appeared significantly lower with HD than with the other diets (*P < 0.001*). Consequently, the sum of *t*-C18:1 isomers was higher for YD (9.5%) and lower with HD (6.8%) than with other diets (*P < 0.001*). The percentage of *c*9*c*12-C18:2 did not differ among treatments and was very low, 1.7% on average. The overall percentage of CLAs appeared to be poorly affected by diets although it tended to be lowered with YD and HD compared to the 2 other diets (*P < 0.10*). The same trend was also noticed for the *c*9t11-CLA and the t9t11-CLA and the proportion of the *t*10c12-CLA was significantly decreased with YD compared with CD (*P < 0.05*). Sums of *t*10 isomers and *t*11 isomers varied in the same manner than *t*10-C18:1 and *t*11-C18:1 respectively, because *t*-C18:1 isomers were much more abundant than CLA and the *t*10/*t*11 isomer ratio was not significantly affected by diets (0.15 on average). Finally, the percentage of *c*9c12*c*15-C18:3 was significantly increased with HD compared with YD or SBD (*P < 0.01*).

EXPERIMENT 2

Initial quantities of unsaturated C18 FA including intermediates of *c*9*c*12-C18:2 BH are presented in Table II: only *t*11 isomers, *t*11-C18:1 and *c*9t11-CLA, were detected in non-incubated cultures, other *c*9*c*12-C18:2 BH intermediates were below the detection level.

After incubation of 6 hours, the FA profiles were affected by the addition of live yeast (Table V). From an analytical point of view, FA determined were the same as in the *in vivo* study but those not present or as traces did not figure in Table V. The percentage of C6:0 measured in flasks supplemented with live yeast tended to be higher than in the control flasks: 1.08 vs. 0.95%. The percentage of C16:1+C17:0anteiso was 33% higher in flasks with live yeast than in control flasks (*P < 0.001*). The percentage of *c*9*c*12-C18:2 tended to be lower in live yeast flasks than in control flasks, and there remained a high amount of C18:2 (59% on average) in all the flasks after the incubation. Percentages of *t*-C18:1 and CLA were very low; their respective sums were <1%, and the sum of *t*-C18:1 was lower than the sum of CLA. The percentage of *t*-C18:1 was higher in live yeast flasks than in control flasks (*P < 0.05*), mainly due to higher proportions of *t*11-C18:1 (*P < 0.05*) and *t*10-C18:1 (*P < 0.10*) in live yeast flasks than in control flasks. The percentage of CLA, including *c*9*t*11-CLA and *t*10*c*12-CLA, was not affected by live yeast addition. As a result, the ratio of *t*10/*t*11 isomers was not affected by live yeast addition.

The rate (V_1) and the efficiency (E_1) of the first step of *c*9*c*12-C18:2 BH, i.e. the isomerisation reaction, were significantly enhanced by live yeast compared to control (Table VI). However, although the rates of the two following reductions were significantly increased by the addition of live yeast, the respective efficiencies of the reactions of reduction remained roughly constant.

Fatty acids	Diets					*P* value
(% of total FA)	CD	SBD	YD	HD	SEM	
C6:0	1.47^{b}	1.71^{b}	2.71^{a}	2.12^{ab}	0.122	**< 0.01**
C7:0	0.15	0.18	0.34	0.21	0.027	< 0.10
C8:0	0.01	0.01	0.02	0.02	0.001	NS
C9:0	0.02	0.02	0.02	0.02	0.001	NS
C10:0	0.03	0.03	0.02	0.03	0.001	NS
C11:0	0.02	0.02	0.02	0.02	0.001	NS
C12:0	0.45^{a}	0.40^{ab}	0.30^{b}	0.36^{ab}	0.018	**< 0.05**
C13:0	0.07^{a}	0.06^{ab}	0.05^{b}	0.06^{ab}	0.002	**< 0.05**
C14:0	1.03	0.96	0.88	1.02	0.022	< 0.10
C15:0	0.51^{b}	0.51^{b}	0.49^{b}	0.60^{a}	0.010	**< 0.05**
C16:0	14.68	14.52	14.10	14.16	0.099	NS
C17:0	0.40	0.40	0.38	0.40	0.004	NS
C18:0	65.72^{ab}	66.33^{ab}	63.99^{b}	67.00^{a}	0.332	**< 0.05**
C13:0 *anteiso*	0.01^{ab}	0.01^{b}	0.01ab	0.02^{a}	0.001	**< 0.05**
C13:0 *iso*	0.05^{b}	0.05^{b}	0.05^{b}	0.08^{a}	0.003	**< 0.001**
C15:0 *anteiso*	0.69^{b}	0.68^{b}	0.82^{ab}	1.00^{a}	0.029	**0.001**
C15:0 *iso*	0.42^{a}	0.29^{b}	0.28^{b}	0.45^{a}	0.011	**< 0.001**
C17:0 *iso*	0.13	0.13	0.13	0.13	0.003	NS
C18:0 *iso*	0.04	0.06	0.05	0.06	0.004	NS
C16:1+C17:0*anteiso*	0.40	0.36	0.39	0.40	0.014	NS
C17 :1	0.02^{b}	0.02^{b}	0.02^{b}	0.03^{a}	0.001	**< 0.001**
*c*9-C18:1	1.84	1.77	1.97	1.73	0.039	NS
*c*11-C18:1	0.60^{a}	0.51^{ab}	0.44^{b}	0.46^{b}	0.016	**< 0.01**
*c*12-C18:1	0.12^{b}	0.11^{b}	0.17^{a}	0.13^{b}	0.004	**< 0.001**
*c*15-C18:1	0.04^{b}	0.04^{b}	0.06a	0.04^{b}	0.001	**< 0.001**
*t*4-C18:1	0.07	0.07	0.08	0.07	0.002	NS
*t*5-C18:1	0.03^{b}	0.04^{b}	0.04^{a}	0.03^{b}	0.001	**< 0.01**
*t*6+*t*7+*t*8-C18:1	0.39^{b}	0.38^{b}	0.50^{a}	0.34^{b}	0.010	**< 0.001**
*t*9-C18:1	0.21^{b}	0.20^{b}	0.27^{a}	0.17^{b}	0.006	**< 0.001**
*t*10-C18:1	0.79^{ab}	0.61^{b}	0.89^{a}	0.57^{b}	0.031	**< 0.01**
*t*11-C18:1	4.29^{bc}	4.58^{ab}	5.13^{a}	3.82^{c}	0.092	**< 0.001**
*t*12-C18:1	0.49^{b}	0.46^{b}	0.62^{a}	0.42^{b}	0.011	**< 0.001**
*t*13+*t*14-C18:1	0.95^{a}	0.85^{a}	0.92^{a}	0.65^{b}	0.024	**< 0.001**
*t*15-C18:1	0.33^{b}	0.31^{b}	0.44^{a}	0.27^{b}	0.009	**< 0.001**
*t*16-C18:1	0.53^{b}	0.49^{bc}	0.60^{a}	0.45^{c}	0.008	**< 0.001**
Sum t-C18:1	***8.10^{ab}***	***8.46^{bc}***	***9.50^{a}***	***6.79^{c}***	***0.190***	***< 0.001***
c9c12-C18:2	1.77	1.63	1.66	1.66	0.067	NS
*t*10*c*12-CLA	0.06^{a}	0.04^{ab}	0.04^{b}	0.05^{ab}	0.003	**< 0.05**
c9c11-CLA	0.02	0.02	0.01	0.01	0.001	NS
c9*t*11-CLA	0.98	0.99	0.86	0.72	0.042	< 0.10
*t*9*t*11-CLA	0.09	0.09	0.08	0.06	0.003	< 0.10
*t*11*c*15-C18:2	0.09	0.09	0.10	0.08	0.003	NS
Sum CLA	*1.14*	*1.13*	*1.00*	*0.85*	*0.045*	***< 0.10***
Sum t10 isomers[1]	***0.85^{ab}***	***0.65^{b}***	***0.93^{a}***	***0.62b***	***0.032***	***< 0.05***
Sum t11 isomers[2]	***5.36^{b}***	***5.66^{ab}***	***6.07^{a}***	***4.60^{c}***	***0.094***	***< 0.001***
t10/t11[3]	***0.17***	***0.12***	***0.16***	***0.15***	***0.007***	***< 0.10***
*c*9*c*12*c*15-C18:3	0.13^{ab}	0.11^{b}	0.13^{b}	0.16^{a}	0.005	**< 0.01**

FA: fatty acids; NS: Not significant; CLA: Conjugated Linoleic Acid; [1]t10-C18:1 + t10c12-CLA; [2]t11-C18:1 + c9t11-CLA + t9t11CLA; [3]sum of t10 isomers / sum of t11 isomers.

TABLE IV: Fatty acid composition of ruminal fluid according to dairy cow diets: CD (control diet), SBD (sodium bicarbonate diet), HD (hay diet) and YD (live yeast diet). Results are expressed as means associated to standard error of the mean (SEM).

Fatty acids	Diets			*P* value
(% of total FA)	Without yeast	With yeast	SEM	
C6:0	0.95	1.08	0.037	< 0.10
C8:0	0.04	0.04	0.002	NS
C10:0	0.01	0.01	0.003	NS
C11:0	0.02	0.02	0.003	NS
C12:0	0.19	0.20	0.007	NS
C13:0	0.03	0.04	0.004	NS
C14:0	0.54	0.58	0.018	NS
C15:0	0.51	0.55	0.019	NS
C16:0	9.67	9.89	0.088	NS
C17:0	0.29	0.31	0.010	NS
C18:0	6.04	6.39	0.114	NS
C15:0 *anteiso*	0.75	0.80	0.029	NS
C15:0 *iso*	0.35	0.39	0.014	NS
C17:0 *iso*	0.08	0.09	0.016	NS
C16:1+C17:0*anteiso*	0.28	0.38	0.008	**< 0.001**
*c*9-C18:1	18.24	18.10	0.088	NS
*c*11-C18:1	0.083	0.083	0.006	NS
*t*6+*t*7+*t*8-C18:1	0.04	0.04	0.003	NS
*t*9-C18:1	0.03	0.04	0.003	NS
*t*10-C18:1	0.07	0.08	0.003	< 0.10
*t*11-C18:1	0.37	0.47	0.019	**< 0.05**
*t*12-C18:1	0.03	0.04	0.005	NS
Sum t-C18:1	***0.54***	***0.66***	***0.026***	**< 0.05**
*c*9*c*12-C18:2	59.44	58.16	0.314	< 0.10
*t*10*c*12-CLA	0.20	0.33	0.041	NS
*c*9*t*11-CLA	0.36	0.49	0.061	NS
Sum CLA	***0.55***	***0.82***	***0.095***	NS
Sum t10 isomers[1]	***0.72***	***0.95***	***0.071***	NS
Sum t11 isomers[2]	***0.27***	***0.42***	***0.044***	NS
t10/t11[3]	***0.43***	***0.43***	***0.038***	NS
*c*9c12*c*15-C18:3	0.64	0.66	0.012	NS

FA: fatty acids; NS: Not significant; CLA: Conjugated Linoleic Acid; [1]t10-C18:1 + t10c12-CLA; [2]t11-C18:1 + c9t11-CLA; [3]sum of t10 isomers / sum of t11 isomers.

TABLE V: Fatty acid composition of ruminal cultures after incubation (6 hours) with or without live yeast. Results are expressed as means associated to standard error of the mean (SEM).

Enzymatic	Diets			*P* value
parameters	Without yeast	With yeast	SEM	
Isomerisation				
V_1 (mg/L/h)	25.49	40.91	2.481	< 0.01
E_1	0.20	0.32	0.020	< 0.01
First reduction				
V_2 (mg/L/h)	24.75	40.01	2.456	< 0.01
Er_2	0.95	0.97	0.007	NS
Second reduction				
V_3 (mg/L/h)	24.18	39.35	2.465	< 0.05
E_3	0.95	0.97	0.011	NS

V and E: rate and efficiency of the reaction calculated as described by TROEGELER-MEYNADIER et al. [34] (cf. Material and Methods); NS: not significant.

TABLE VI: Rates (V, mg/L/h) and efficiencies (E) of the three reactions of linoleic acid biohydrogenation after incubation for 6 hours with or without live yeast.

Discussion

Although CD contained more than 60% concentrates (on a DM basis), it did not induce a subacute ruminal acidosis probably because of a low DM intake. Neither live yeast, nor sodium bicarbonate (SB), nor HD had an effect on ruminal pH. An effect of live yeast on ruminal pH was expected when cows are fed with a high concentrate diet inducing subacute ruminal acidosis [20]. Similarly, a strong reducing ruminal milieu was observed with CD (E_h = −202 mV) which was not influenced by HD, SB or live yeast supplementation. These observations contrasted with results obtained by MARDEN *et al*. [20] who observed a more reducing status of the rumen with live yeast: −149 mV vs. −115 mV for the control diet. In this study, it seems that live yeast failed to improve the ruminal reducing power when the redox status induced by the control diet was around −200 mV. In the same way, neither live yeast nor SB had a significant effect on fermentative ruminal parameters (Table III). On the contrary, replacing part of maize silage by hay induced a lower ruminal concentration of butyrate and propionate and a higher concentration in N-NH_3 (*P < 0.05*) as previously observed *in vitro* by CALSAMIGLIA *et al*. [4].

Live yeast supplementation increased ruminal proportions of *t*-C18:1 and decreased the proportion of C18:0, suggesting an inhibition of the last step of *c*9*c*12-C18:2 BH *i.e.* the second reduction into C18:0. As live yeast is known to modulate ruminal bacterial populations [25], it could be hypothesized that some bacteria involved in C18:0 production would be affected by the presence of live yeast. Unfortunately, bacteria involved in C18:1 reduction to C18:0 are not yet well known therefore preventing further discussion on the effects of live yeast. On the other hand, the second reduction reaction could also be inhibited by an excess of substrates i.e. *t*-C18:1 as already stated by TROEGELER-MEYNADIER *et al*. [34]. The in vivo experiment did not provide enough information to conclude on the possible inhibition of BH because *c*9*c*12-C18:2 BH was very efficient as shown by high ruminal content of C18:0 (66% on average) and low content of *c*9*c*12-C18:2 (2% on average). The *in vitro* disappearance of *c*9*c*12-C18:2 was lower than that observed by TROEGELER-MEYNADIER *et al*. [34] with a 7 pH-buffer (E1 = 34% and E1 = 68% after 4 and 8 hours of incubation, respectively), and in accordance with that recorded with a 6 pH-buffer (E1 = 20% and E1 = 41% after 4 and 8 hours of incubation, respectively). The low percentage of disappearance could be due to the low pH as already shown by TROGELER-MEYNADIER *et al*. [34], or to insufficient activity of biohydrogenating bacteria. Our donor cow received a hay diet, so that lactate consuming bacteria, like *M. elsdenii*, which are favoured by high grain-based diets [15], probably represented a low proportion of bacteria present in the inoculums. Instead, fibrolytic bacteria would be suspected to be the most predominant bacterial species in the ruminal sampled inoculum on our donor cows. With the introduction of grain into the incubation medium, the population of *B. fibrisolvens* could be expected to rapidly decline since starch is not the adequate substrate for their growth [15].

Nevertheless, the rate and efficiency of isomerisation (Table VI) obtained with live yeast were 1.6 higher than in control flasks (40.9 *vs.* 25.5 mg/L/h for the rate and 32% *vs.* 20% for the efficiency), leading to a lower percentage of *c*9*c*12-C18:2 in live yeast flasks than in control. This more rapid disappearance of *c*9*c*12-C18:2 could be the result of live yeast FA uptake for oxidative metabolism, as already described by Shüller [28], but this is unlikely to occur in strong ruminal reducing conditions *in vitro* [12]. The rates of both reductions (Table VI) were also increased by live yeast but efficiencies were not: the low reduction rate observed in control flasks were mainly due to a lack of substrate produced by isomerisation.

Thus, the accumulation of *t*-C18:1 (*P = 0.034*) observed with live yeast could in part be explained by an improvement of all the pathways of isomerisation of *c*9*c*12-18:2 which generated a large amount of intermediates able to induce an inhibition or a saturation of the enzyme activity of bacteria involved in the second reduction step [34]. The rapid isomerisation observed with live yeast was more likely to originate from an effect on bacterial communities than from a direct effect on enzymatic processes, since live yeast are known to affect bacterial populations [25]. Indeed, the amount of *t*11-C18:1 was increased by live yeast and *t*11-C18:1 is known to be produced by *B. fibrisolvens*, a fibrolytic bacterium. In addition, PINLOCHE *et al*. [25] observed an increase in the taxa representing fibrolytic bacteria in live yeast fed dairy cows. Thus, live yeast may act by favouring fibrolytic bacteria involved in the *t*11 pathway of ruminal *c*9*c*12-C18:2 BH. The content of *t*10 isomers was increased in vivo and tended to be increased *in vitro* by live yeast. Live yeast may also favour supposed *t*10 producing bacteria: *M. elsdenii* [27] and/or *P. acnes*, an *Actinobacteria* [25]. As a consequence, the addition of live yeast to the media (experiment 2) or to the diet (experiment 1) was able to modulate *c*9*c*12-C18:2 BH without modifying the *t*10/*t*11 isomer ratio. Furthermore, in vivo, an increase in *c*12-C18:1 with YD was observed in parallel with an increase of *t*10-C18:1 as already stated by SHINGFIELD *et al*. [29]. Live yeast also resulted in increased proportions of *c*15-C18:1, *t*5-C18:1, *t*6+*t*7+*t*8-C18:1, *t*9-C18:1, *t*12-C18:1, *t*15-C18:1, *t*16-C18:1, but the bacteria which produce them are at the present time unidentified.

SB has been demonstrated to favour t11-isomers and decrease t10 isomers in ruminal content presumably by preventing a decrease in rumen pH [35]. In the present in vivo study, CD was not acidogenic enough to result in a high production of t10 isomers and an inhibition in the production of *t*11 isomers, so that the effects of SB on *t*10-C18:1 and *t*11-C18:1 were low. Except for *t*11-C18:1 and *t*13+*t*14-C18:1, all the other *t*-C18:1 isomer percentages, measured with SBD were significantly lower than with YD. Live yeast seemed to favour *t*-C18:1 accumulation more than SB because of increased inhibition of the second reduction step: there was less C18:0 produce with YD than SBD (*P = 0.078*). It would be interesting to test this hypothesis in more drastic acidogenic ruminal conditions.

Regarding introduction of fibre in a high concentrate diet enriched with FA, it has been demonstrated that the addition of straw leads to an increase in C18:0 ruminal contents and a decrease in *t*10-C18:1, *t*11-C18:1 and *c*9*t*11-CLA contents [6]. In the present study, replacement of part of the maize

silage by a coarse chopped hay also led to a better complete ruminal BH. However, the accumulation of intermediates of BH such as *t*11-C18:1 or *c*9*t*11-CLA is preferred when enriching milk in *c*9*t*11-CLA. Therefore, the addition of fibre to an FA enriched high concentrate diet was less effective than a live yeast supplement which significantly favoured intermediates of BH. In the present study, all the *t*-C18:1 even *t*11-C18:1 contents were increased to a greater extent with live yeast than with HD: +40% for *t*-C18:1 and +34% for *t*11-C18:1. With HD there is no inhibition of the second reduction step in BH, leading to significantly higher ruminal contents of C18:0 with HD than with YD and no ruminal accumulation of intermediates of *c*9*c*12-C18:2 BH. When the proportions of *t*11 isomers are concerned, the *B. fibrisolvens* population could be suspected to be enhanced more with live yeast than with fibre as underlined by a significantly higher butyrate concentration after YD than HD [7]. So coarse chopped hay and live yeast may favour the development of different fibrolytic populations with varying capacities for BH.

To conclude, live yeast was a good modulator of ruminal c9c12-C18:2 BH by enhancing production of BH intermediates. Among BH intermediates, all *t*-C18:1 (except *t*13+*t*14-C18:1) *c*12-C18:1 and *c*15-C18:1 were increased by live yeast which suggested an inhibition of the second reduction reaction by an improvement of the isomerisation of polyunsaturated fatty acids (PUFA). Therefore, the inhibition of the second reduction step of BH induced by live yeast would be interesting when trying to increase ruminal *t*11-C18:1 accumulation and as a consequence *c*9*t*11-CLA in dairy cow milk. These promising results must be confirmed in the future with more acidogenic diets.

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References

1. - AFNOR : Dosage de l'azote en vue du calcul de la teneur en protéines brutes. NF V18-100, in *Collection de normes françaises et de méthodes communautaires. Aliments des Animaux. Méthodes d'Analyse*. 1977, Paris.
2. - AFNOR : Détermination de la teneur en amidon. NF V 18-121, in *Collection de normes françaises et de méthodes communautaires. Aliments des Animaux. Méthodes d'Analyse*. 1977, Paris.
3. - AFNOR : Détermination séquentielle des constituants pariétaux. NF V 18-122, in *Collection de normes françaises et de méthodes communautaires. Aliments des Animaux. Méthodes d'Analyse*. 1997, Paris.
4. - CALSAMIGLIA S., CARDOZO P.W., FERRET A., BACH A.: Changes in rumen microbial fermentation are due to a combined effect of type of diet and pH. *J. Anim. Sci*., 2008, **86**, 702-711.
5. - CHAUCHEYRAS F., MILLET L., MICHALET-DOREAU B., FONTY G., BERTIN G., GOUET P.: Effect of the addition of Levucell Sc on the rumen microflora of sheep during adaptation to high starch diets. in *Rumen microbial ecosystem symposium*. 1997. Aberdeen, UK.
6. - DANG VAN Q.C., FOCANT M., DESWYSEN D., MIGNOLET E., TURU C., POTTIER I., FROIDMONT E., LARONDELLE Y.: Influence of an increase in diet structure on milk conjugated linoleic acid content of cows fed extruded linseed. *Animal*, 2008, **2**, 1538-1547.
7. - DEHORITY B.A.: *Rumen microbiology*. 372 pages. Nottingham University Press, UK.
8. - DESNOYERS M., GIGER-REVERDIN S., BERTIN G., DUVAUX-PONTER C., SAUVANT D.: Meta-analysis of the influence of Saccharomyces cerevisiae supplementation on ruminal parameters and milk production of ruminants. *J. Dairy Sci.*, 2009, **92**, 1620-1632.
9. - GRIINARI J.M., CORL B.A., LACY S.H., CHOUINARD P.Y., NURMELA K.V.V., BAUMAN D.E.: Conjugated linoleic acid is synthesized endogenously in lactating dairy cows by Delta(9)-desaturase. *J. Nutr*., 2000, **130**, 2285-2291.
10. - HOUBA V.J.G., Novozamsky I., Uittenbogaard J., Van Der Lee J.J.: Automatic determination of total soluble nitrogen in soil extracts, *Landwirtsch Forschung*. 1987.
11. - IP M.M., MCGEE S.O., MASSO-WELCH P.A., IP C., MENG X.J., OU L.H., SHOEMAKER S.F.: The *t*10,*c*12 isomer of conjugated linoleic acid stimulates mammary tumorigenesis in transgenic mice over-expressing erbB2 in the mammary epithelium. *Carcinogenesis*, 2007, **28**, 1269-1276.
12. - JULIEN C., TROEGELER-MEYNADIER A., MARDEN J.P., ENJALBERT F., BAYOURTHE C.: *In vivo* and *In Vitro* measurements of ruminal redox potential: A comparative study. In: *Joint Annual Meeting*. 2009. Montreal, Québec, Canada.
13. - KALSHEUR K.F., TETER B.B., PIPEROVA L.S., ERDMAN, R.A.: Effect of dietary forage concentration and buffer addition on duodenal flow of trans-C18:1 fatty acids and milk fat production in dairy cows. *J. Dairy Sci.*, 1997, **80**, 2104- 2114.
14. - KIM Y.J., LIU R.H., RYCHLIK J.L., RUSSELL J.B.: The enrichment of a ruminal bacterium (*Megasphaera elsdenii* YJ-4) that produces the trans-10, cis-12 isomer of conjugated linoleic acid. *J. Appl. Microbiol.*, 2002, **92**, 976-982.
15. - KLIEVE A.V., HENNESSY D., OUWERKERK D., FORSTER R.J., MACKIE R.J., ATTWOOD G.T.: Establishing populations of *Megasphaera elsdenii* YE 34 and *Butyvibrio fibrisolvens* YE 44 in the rumen of cattle fed high grain diets. *J. Appl. Microbiol*., 2003, **95**, 621-630.
16. - KROM M.D.: Spectrophotometric determination of ammonia. *Analyst*, 1980, **105**, 305-316.
17. - LILA Z.A., MOHAMMED N., TAKAHASHI T., TABATA M., YASUI T., KURIHARA M., KANDA S., ITABASHI H.: Increase of ruminal fiber digestion by cellobiose and a twin strain of *Saccharomyces cerevisiae* live cells in vitro. *Anim. Sci. J.*, 2006, **77**, 407-413.
18. - MAIA M.R.G., CHAUDHARY L.C., FIGUERES L., WALLACE R.J.: Metabolism of polyunsaturated fatty acids and their toxicity to the microflora of the rumen. *Antonie Van Leeuwenhoek*, 2007, 91, 303-314.
19. - MARDEN J.P., BAYOURTHE C., ENJALBERT F., MONCOULON R.: A new device for measuring kinetics of ruminal pH and redox potential in dairy cows. *J. Dairy Sci*., 2005, **88**, 277-281.
20. - MARDEN J.P., JULIEN C., MONTEILS V., AUCLAIR E., MONCOULON R., BAYOURTHE C.: How does live yeast differ from sodium bicarbonate to stabilize ruminal pH in high-yielding dairy cows? *J. Dairy Sci*., 2008, **91**, 3528-3535.
21. - MOON C.D., PACHECO D.M., KELLY W.J., LEAHY S.C., LI D., KOPECNY J., ATTWOOD G.T.: Reclassification of *Clostridium proteoclasticum* as *Butyrivibrio proteoclasticus* comb. nov., a butyrateproducing ruminal bacterium. *Int. J. Syst. Evol. Microbiol*., 2008, **58**, 2041-2045.
22. - NISBET D.J., MARTIN S.A.: Effect of a *Saccharomyces cerevisiae* culture on lactate utilization by the ruminal bacterium *Selenomonas ruminantium. J. Anim. Sci*., 1991, **69**, 4628-4633.
23. - PARIZA M.W., PARK Y., COOK M.E.: The biologically active isomers of conjugated linoleic acid. *Prog. Lipid. Res*., 2001, **40**, 283-298.
24. - PARK P.W., GOINS R.E.: *In situ* preparation of fatty acid methyl esters for analysis of fatty acid composition in foods. *J. Food Sci.*, 1994, **59**, 1262-1266.
25. - PINLOCHE E., MCEVAN N., MARDEN J.P., BAYOURTHE C., MONCOULON R., NEWBOLD C.J.: Effect of yeast on the bacterial diversity of the rumen. in 6th INRA-RRI Symposium. *Gut Microbiology*. 2008. Clermont-Ferrand, France.

26. - PLAYNE M.J.: Determination of ethanol, volatile fatty acids, lactic acid and succinic acid in fermentation liquids by gas chromatography. *J. Sci. Food Agric*., 1985, **36**, 638-644.

27. - ROSSI F., DI LUCCIA A., VINCENTI D., COCCONCELLI P.S.: Effects of peptidic fraction from *Saccharomyces cerevisiae* culture on growth and metabolism of ruminal bacteria *Megasphaera elsdenii. Anim. Res*., 2004, **53**, 177-186.

28. - SCHULLER H.J.: Transcriptional control of nonfermentative metabolism in the yeast *Saccharomyces cerevisiae. Curr. Genet.*, 2003, **43**, 139-160.

29. - SHINGFIELD K.J., AHVENJARVI S., TOIVONEN V., VANHATALO A., HUHTANEN P., GRIINARI J.M.: Effect of incremental levels of sunflower-seed oil in the diet on ruminal lipid metabolism in lactating cows. *Br. J. Nutr*., 2008, **99**, 971-983.

30. - STREETER M.N., WAGNER D.G., HIBBERT C.A., OWENS F.N.: Comparison of corn with four sorghum grain hybrids: Site and extent of digestion in steers. *J. Anim. Sci*., 1990, **68**, 3429-3440.

31. - TRICON S., BURDGE G.C., KEW S., BANERJEE T., RUSSELL J.J., JONES E.L., GRIMBLE R.F., WILLIAMS C.M., YAQOOB P., CALDER P.C.: Opposing effects of cis-9,trans-11 and trans-10,cis-12 conjugated linoleic acid on blood lipids in healthy humans. *Am. J. Clin. Nutr.*, 2004, **80**, 614-620.

32. - TROEGELER-MEYNADIER A., ENJALBERT F.: Les acides linoléiques conjugués : 1. Interêts biologiques en nutrition. *Rev. Méd. Vét.*, 2005, **156**, 207-216.

33. - TROEGELER-MEYNADIER A., ENJALBERT F.: Les acides linoléiques conjugués : 2. Origines et effets sur les productions animales. *Rev. Méd. Vét*., 2005, **156**, 281-288.

34. - TROEGELER-MEYNADIER A., BRET-BENNIS L., ENJALBERT F.: Rates and efficiencies of reactions of ruminal biohydrogenation of linoleic acid according to pH and polyunsaturated fatty acids concentrations. *Rep. Nutr. Dev*., 2006, **46**, 713-724.

35. - TROEGELER-MEYNADIER A., NICOT M.C., ENJALBERT F.: Effects of fat source and dietary sodium bicarbonate plus straw on the conjugated linoleic acid content of milk of dairy cows. *Arch. Anim. Nutr.*, 2007, **61**, 406-415.

36. - TURPEINEN A.M., MUTANEN M., ARO A., SALMINEN I., BASU S., PALMQUIST D.L., GRIINARI J.M.: Bioconversion of vaccenic acid to conjugated linoleic acid in humans. *Am. J. Clin. Nutr.*, 2002, **76**, 504-510.

37. - VERDOUV H., VAN ECHTELD C.J.A., DEKKERS E.M.J.: Ammonia determination based on indophenol formation with sodium salicylate. *Water Res.*, 1977, **12**, 399-402.

38. - VERHULST A., JANSSEN G., PARMENTIER G., EYSSEN H.: Isomerization of polyunsaturated long-chain fatty–acids by *Propioibacteria. Syst. Appl. Microbiol.*, 1987, **9**, 12-15.

39. - WILSON T.A., NICOLOSI R.J., SAATI A., KOTYLA T., KRITCHEVSKY D.: Conjugated linoleic acid isomers reduce blood cholesterol levels but not aortic cholesterol accumulation in hypercholesterolemic hamsters. *Lipids*, 2006, **41**, 41-48.