Effects of dietary Saccharomyces cerevisiae live yeast culture supplementation on ruminal digestion and protozoa count in rams fed with diets with low or high ratio forage/concentrate

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SUMMARY

In this study, 4 male one year old Kivircik rams with permanent rumen cannula were used. Feeding of the animals was performed according to 4 x 4 Latin square designs with 20 days periods (15 days adaptation period, 5 days collection period). Animals were fed with two different diet types: Diet 1 consisted of 70 % alfalfa hay and 30 % concentrate diet while Diet 2 consisted of 30 % alfalfa hay and 70 % concentrate. Diet 1 and 2 were supplemented with or without a daily dose of 4 g Yea-Sacc1026 (2.109 CFU/day) Saccharomyces cerevisiae live yeast culture (YS). Rumen contents collected at 0 (before morning feeding), 2 and 4 h (after morning feeding) on days 1 and 5 in each collection period were analyzed for pH, protozoa counts, total volatile fatty acid (VFA) concentration, NH3-N, ruminal cellulolytic activity. When YS was added to the forage-enriched ration (Diet 1), ruminal pH was significantly decreased at 2 hours (P < 0.05) and in parallel, ruminal VFA concentrations tended to increase (P < 0.01). Ruminal NH3-N concentrations were significantly increased at 4 hours (P < 0.05) by dietary YS supplementation whatever the ratio forage/concentrate of the diet. By contrast, protozoa counts and cellulolytic activity were not significantly affected in the presence of YS. These results suggest that the ruminal fermentation would be more easily affected by dietary YS addition when rams consumed a diet rich in forage.

Keywords: Rams, Saccharomyces cerevisiae, ruminal protozoa, ruminal digestion.

RÉSUMÉ

Effets de l’addition de levures, Saccharomyces cerevisiae, sur la digestion et la population de protozoaires du rumen chez des béliers nourris avec des rations dont le rapport fourrage/concentré est variable.

Quatre béliers Kivircik âgés de 1 an, portant une canule ruminale, ont été utilisés dans cette étude selon un protocole en carré latin avec des périodes de 20 jours (15 jours d’adaptation et 5 jours de prélèvements). Ils ont reçu 2 régimes alimentaires différents : le régime 1 était constitué par 70 % de foin de luzerne et 30 % de concentrés tandis que le régime 2 était constitué par 30 % de foin de luzerne et 70 % de concentrés. Dans ces 2 régimes, une dose quotidienne de 4 g de cultures vivantes de Saccharomyces cerevisiae (Yea-Sacc1026, 2.109 CFU/jour, YS) a été ou non incorporée. Les contenus ruminaux collectés avant (0h) et 2 et 4 heures après le repas le 1er et le 5e jours de chaque période de prélèvements ont été analysés (détermination du pH, de la numération de protozoaires, des concentrations de NH3-N et des acides gras volatiles et de l’activité cellulolytique). Lorsque les levures ont été ajoutées au régime 1 (riche en foin), le pH du rumen a significativement diminué à 2h (P < 0.05) et parallèlement les concentrations en AGV ont eu tendance à augmenter (P < 0.1). A 4h, une élévation significative des concentrations en NH3-N a été mise en évidence lorsque les levures ont été incorporées au régime quelqu’en soit le rapport foin/concentré (P < 0.05). En revanche, la population des protozoaires et l’activité cellulolytique n’ont pas été significativement modifiées en présence des levures. Ces résultats suggèrent que la digestion ruminale des béliers pourrait être plus facilement modulée par l’addition de levures dans la ration lorsque celle-ci est riche en foin.

Mots-clés : Bélier, Saccharomyces cerevisiae, rumen, protozoaires, digestion.

Introduction

For many years, ruminant nutritionists and microbiologists have been interested in manipulating the microbial ecosystem of the rumen to improve production efficiency by domestic ruminants. Based on growing concern over the use of antibiotics and other growth promoters in the animal feed industry, interest in the effects of microbial feed additives on animal performance has increased during the past 10 to 20 years. Addition of Saccharomyces cerevisiae cultures to ruminant diets has improved fibre digestibility and stimulated cellulolytic bacteria [6, 29 - 31], but also the dietary Saccharomyces cerevisiae supplementation has increased the ruminal pH [30], the protozoa count [12], and total concentrations of volatile fatty acids, and decreased the NH3-N [6, 8]. The beneficial effects of dietary yeast culture supplementation on the proportions of the different protozoa types in rumen, leading to positive effects on cellulose digestibility and to systemic metabolic consequences (characterized by increases of serum total protein, urea and calcium concentrations and decrease of triglyceride concentrations) has been recently evidenced in rams [10]. But, in other studies, no influence or contrary results were obtained [3, 5]. Consequently, the highly variable effects of live Saccharomyces cerevisiae cultures could be associated with the respective proportions of forage and concentrate of diets [9], to the animal specie and to the sampling times of rumen fluid [8]. Therefore, the objective of this experiment was to inves-
tigate the influence of the ratio forage/concentrate of the diet on the potential dietary Saccharomyces cerevisiae supplementation efficiency by analysing ruminal parameters (pH, NH3-N, volatile fatty acid concentrations, protozoa counts and cellulolytic activity) in Kivircik rams at different sampling times relative to meal.

Materials and methods

ANIMALS AND FEED

Four male one year old Kivircik rams (average weight of 50 kg) with permanent rumen cannula were used. Feeding of the animals was performed according to 4 x 4 Latin square design with 20 days periods (15 days adaptation period, 5 days collection period). Animals were fed with two different diet types characterized by the forage (alfalfa hay)/ concentrate ratio (Diet 1 with a ration of 7/3 and diet 2 with a ratio of 3/7). A daily dose of 4 g Ye-Sacc™ (Alltech, Nicholasville; 5x10⁹ CFU/g, Saccharomyces cerevisiae live yeast culture, YS) was or was not added to the 2 diets. Chemical compositions of the diets are given in Table I and formulation of concentrate diet in Table II. Chemical analyses of diets were carried out according to AOAC [1]. Diet was designed to meet 1.25 times of NRC [20] maintenance requirements for sheep. Diets were divided into two equal parts and fed at 09.00 am and 16.00 pm. The sheep were housed individually in loose pens during this period.

RUMEN SAMPLING

Rumen fluid samples were taken through the rumen cannula at 0 (before morning feeding, 9.00 a.m.), 2 and 4 (after morning feeding, 11.00 a.m. and 13.00 p.m., respectively) hours on days 1 and 5 of collection period in each period. The pH of each sample was determined immediately after morning feeding, 11.00 a.m. and 13.00 p.m., respectively) hours on days 1 and 5 of collection period in each period. The pH of each sample was determined immediately with an electronic pH meter. Ammonia nitrogen (NH3-N) and total volatile fatty acids (VFA) concentrations of ruminal fluid were measured according to steam distillation method described by MARKHAM [15]. One ml of rumen fluid was mixed with 49 ml of rumen protozoa counting solution (2.02 % formalin and 15.15 % glycerol) to determine the counts of rumen protozoa. Diluted ruminal fluid samples were used for counting cells with the help of Fuchs Rosenthal counting chamber by the method of BOYNE et al. [4]. Cellulose activity into the rumen was measured by loss of weight of cotton thread inoculated in sacco during 24 hours in each period [23].

STATISTICAL ANALYSIS

Data were analyzed using a 4 x 4 Latin-square design model. Four combinations (2 diets, with or without yeast) were used as a single factor. Analysis of variance with 2 independent factors (diet and yeast) and their interaction were studied [26]. Differences between means were identified by

<table>
<thead>
<tr>
<th></th>
<th>Diet 1</th>
<th>Diet 2</th>
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<tbody>
<tr>
<td>Alfalfa hay %</td>
<td>70</td>
<td>30</td>
</tr>
<tr>
<td>Concentrate %</td>
<td>30</td>
<td>70</td>
</tr>
<tr>
<td>Dry Matter</td>
<td>88.76</td>
<td>89.80</td>
</tr>
<tr>
<td>Crude Protein %</td>
<td>21.51</td>
<td>20.28</td>
</tr>
<tr>
<td>Ether Extract %</td>
<td>2.90</td>
<td>3.56</td>
</tr>
<tr>
<td>Organic Matter %</td>
<td>88.91</td>
<td>91.15</td>
</tr>
<tr>
<td>¹NDF %*</td>
<td>41.96</td>
<td>35.10</td>
</tr>
</tbody>
</table>

* Bases of % Dry Matter, ¹Neutral detergent fiber.

Tukey’s Test [27]. Data showing P < 0.05 were significant but trends (P < 0.1) are also noted and discussed.

Results and discussion

The ruminal pH before feeding was significantly lower in rams fed with the diet 2 (concentrate enriched diet) compared to rams fed with the diet 1 (forage enriched diet) (p < 0.05) (Figure 1). The ruminal pH decreased after meal (at 2 and 4h) in the both cases but it remained significantly higher when a forage enriched ration (diet 1) was used (at 2h and 4h, diet 1 vs. diet 2 : p < 0.05). The YS addition to the diet 1 significantly amplified the ruminal pH decrease particularly 2h after feeding (p < 0.05), whereas the effect was less pronounced (p < 0.1) in rams fed with the diet 2 (Figure 1). At 4h, ruminal pH tended to be lower in rams fed with YS supplemented diet 1 compared to those fed with non supplemented diet 1 (p < 0.1) whereas it was similar in rams receiving the diet 2 with or without YS (Figure 1). A tendency for pH to decrease after supplementation by YS was also observed in the experiment of ENJALBERT et al. [8]. Previous experiments have shown a slow decrease of ruminal pH after feeding With YS supplemented rations containing 50 % barley, but the drop of pH under 6.0 was delayed on sheep [17] and prevented in dairy cows receiving a complete diet [30]. This effect may be due to

**Table I.** Composition and nutrients content of diets (% DM).

<table>
<thead>
<tr>
<th></th>
<th>Concentrate diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barley</td>
<td>69.20</td>
</tr>
<tr>
<td>Corn</td>
<td>10.00</td>
</tr>
<tr>
<td>Sunflower meal</td>
<td>17.60</td>
</tr>
<tr>
<td>Limestones</td>
<td>2.50</td>
</tr>
<tr>
<td>Salt (NaCl)</td>
<td>0.60</td>
</tr>
<tr>
<td>Vitamin-Mineral Premix a</td>
<td>0.10</td>
</tr>
</tbody>
</table>

aCP : Crude Protein ; bprovided per kilogram of Vitamin-Mineral Premix : Vit A 15.000 000 IU, Vit D3 3.000 000 IU, Vit E 30 000 mg, Mn 50 000 mg, Fe 50 000 mg, Zn 50 000 mg, Cu 10000 mg, I 800 mg, Co 150 mg, Se 150 mg.

**Table II.** Formulation of concentrate diet (% as fed).
The addition of YS to diets did not induce any significant variation of total VFA concentrations in ruminal fluid [11, 33]. Nevertheless, the slight increases of ruminal VFA concentrations observed in the present study especially when the forage rich ration was supplied by YS would be linked to a microbial activity improvement.

In this trial, the ruminal concentrations of NH$_3$-N were higher when non supplemented diet 2 was given to rams compared to the non supplemented diet 1 (p < 0.05). In the both cases, this parameter slightly increased 2h after meal then again decreased at 4h (Figure 3). When the both diets were supplemented by YS, the variations of NH$_3$-N concentrations were exacerbated (p < 0.1 at 2h) and NH$_3$-N values remained significantly higher 4h after feeding than the respective control values (at 4h, diet vs. Diet + YS : p < 0.05) (Figure 3). These results were in agreement with other studies [7, 16, 18] which exhibited slight increases of NH$_3$-N contents in rumen after feeding with dietary YS supplemented diets. This variation could be due to an increase of proteolysis and of protein deamination by micro organisms [28]. More information is needed on this subject because most of the studies involve changes on cellulolytic bacteria population [21] and protozoa [18, 24]. On the opposite, other reports stated that ruminal NH$_3$-N content seemed to be depressed by dietary YS supplementation [6, 8]. A lower degradation of dietary proteins (attested by an increased duodenal flow of non degraded feed nitrogen [25]) would support these observations. And finally, other researchers did not evidence any variation of the NH$_3$-N content due to YS supplementation [2, 5]. Furthermore, HARRISON et al. [11] also showed a lower variability in NH3-N of ruminal fluid when YC was added to the diet.

### Table III

<table>
<thead>
<tr>
<th>Items</th>
<th>Sampling times (h)</th>
<th>Treatments</th>
<th>S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Diet 1</td>
<td>Diet 1 + YS</td>
</tr>
<tr>
<td>Protozoa count ($10^3$)</td>
<td>0</td>
<td>1168.75</td>
<td>866.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>827.00</td>
<td>463.50</td>
</tr>
<tr>
<td></td>
<td></td>
<td>d</td>
<td>d</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>868.50</td>
<td>519.50</td>
</tr>
<tr>
<td>Cellulose activity (%)</td>
<td>24</td>
<td>60.03</td>
<td>63.48</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ed</td>
<td>c</td>
</tr>
</tbody>
</table>

Different superscripts (c, d) in the same lane indicate significant differences (P < 0.05) between groups. *Cellulose activity into the rumen was measured by loss of weight of cotton thread inoculated in sacco during 24 hours.

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**Figure 1.** Evolution of the ruminal pH according to the sampling time (0, 2 and 4 hours after feeding) in rams fed with 2 different diets (Diet 1: 70 % alfalfa hay and 30 % concentrate; Diet 2: 30 % alfalfa hay and 70 % concentrate) supplemented or not with live yeast *Saccharomyces cerevisiae* cultures (YS, 4 g/j, i.e. 20x10$^5$ CFU).

Different superscripts (a, b, c, d, e) indicate significant differences ($^{a,b}$ P < 0.01, $^{c,d,e}$ P < 0.05) between groups.
Although the differences were not statistically significant because of the great dispersion of the values, the YS addition to diets tended to depress protozoa counts, particularly 2 hours after feeding (Table III). At 4h, this effect was markedly attenuated. These results were in agreement with the study of CORANO et al. [5] which observed a tendency for the total protozoa population to decrease \((p = 0.13)\) in the presence of \textit{Saccharomyces cerevisiae}. By contrast, some authors reported elevations of total protozoa counts when animals were fed with low quality diets [24] but the influence of \textit{Saccharomyces cerevisiae} on the total population was much debated [18].

According to WILLIAMS [29], yeast cultures may provide factors which stimulate rumen cellulytic and proteolytic bacteria especially when high concentrate (> 50%) diets are given. Yeast supplement may stimulate cellulytic bacteria and improve fibre digestibility [6] and digestibility of proteins and cellulose were improved in ruminant fed with supplemental yeast [32]. Also, in my previous study [10] addition of YC to diet has significantly modified the proportions of the different protozoa types, and has improved ruminal cellulytic activity in a dose dependent way. However, in the present study, albeit this parameter seemed to be enhanced in rams receiving YS supplemented diet 2, there was no statistically significant effect of YS on the ruminal cellulytic activity (Table III).

Many investigators have attributed the beneficial effects of yeast culture directly to changes in the ruminal fermentation and in the microbial population in the digestive tract [6, 8, 29 - 31]. Several studies demonstrated that yeast culture supplementation can influence digestive processes in the rumen [14, 22]. In these studies, the initial rate of digestion was readily influenced by the addition of YS to the diets of ruminants. Since feed intake is often considered to be a function of the initial rate of fibre digestion, early stimulation of ruminal activity can be expected to have a major impact on feed consumption and can provide a driving force for improved animal performance. Such studies suggest an important role of yeast culture supplementation for digestion in animals receiving high forage diets. In this study, ruminal pH decreased and \(\text{NH}_3\) increased in rams consumed diets rich in forage and supplied with YS, and a tendency for VFA to increase was also evidenced. The changes in ruminal activity could result from an increase of proteolysis and of protein deamination by micro organisms. It can be concluded that the addition of YS would play a more important role in rams consumed diets rich in forage than in those consumed diets rich in concentrate. However, more studies would be necessary to obtain definitive evidence on diet - specific advantages of supplementing yeast.

Acknowledgements

This study was financed by research fund of Uludag University in Bursa in Turkey.

References


\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure2.png}
\caption{Figure 2. Evolution of the total volatile fatty acid (VFA) concentrations according to the sampling time (0, 2 and 4 hours after feeding) in rumin of rams fed with 2 different diets (Diet 1: 70 % alfalfa hay and 30 % concentrate; Diet 2: 30 % alfalfa hay and 70 % concentrate) supplemented or not with live yeast \textit{Saccharomyces cerevisiae} cultures (YS, 4 g/j, i.e. 2x10^9 CFU). Different superscripts (a, b) indicate significant differences \((P < 0.1)\) between groups.}
\end{figure}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure3.png}
\caption{Figure 3. Evolution of the \(\text{NH}_3\)-N concentrations according to the sampling time (0, 2 and 4 hours after feeding) in rumin of rams fed with 2 different diets (Diet 1: 70 % alfalfa hay and 30 % concentrate; Diet 2: 30 % alfalfa hay and 70 % concentrate) supplemented or not with live yeast \textit{Saccharomyces cerevisiae} cultures (YS, 4 g/j, i.e. 2x10^9 CFU). Different superscripts (a, b, c, d, e) indicate significant differences \((a, b P < 0.1, c, d, e P < 0.05)\) between groups.}
\end{figure}


