

Predicting the Impact of a Live Yeast Strain on Rumen Kinetics and Ration Formulation

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Introduction

Yeast additives have been fed to animals for more than 100 years. Although there are about 500 different species of yeast, the most common one fed to cows is *Saccharomyces cerevisiae*. Strains of *Saccharomyces cerevisiae* on the market today vary widely. The American Association of Feed Control Officials defines an **active dry yeast** as containing no less than 15 billion live yeast cells (cfu) per gram, being dried to preserve its fermenting power, and containing no filler product (e.g Levucell SC I-1077). Unlike active dry yeast, **yeast culture products** are defined as containing yeast and the media on which it was grown. Some yeast culture products contain live cells, where as other yeast culture products do not guarantee any live yeast cells in their product. They may also contain a carrier such as rice hulls. They rely on dead yeast cells, the media the yeast was grown on, and metabolites made by the yeast cell during the manufacturer's fermentation process to have a positive effect on rumen fermentation.

This paper will focus primarily on the active dry yeast product Levucell SC I-1077 (20 billion cfu/gram) from Lallemand Animal Nutrition. Its mode of action has been intensively researched as described below and many cow research studies have been completed so far. Research with other yeast additives is also highlighted in this paper. Modes of action and cow responses may differ among commercial products.

Modes of action of *Saccharomyces cerevisiae* (SC I-1077) in the rumen

In the rumen, feed digestion involves numerous and complex interactions between different microbial anaerobic communities (bacteria, fungi, protozoa), which enable the animal to remarkably benefit from the diet. Nevertheless in practice several factors (early weaning, high concentrate diets, brutal feed transition, environmental stress, etc...) may affect the microbial balance and digestive troubles can occur (ruminal acidosis, bloat, etc) which can lead to an alteration of health and performance.

Live yeasts (*Saccharomyces cerevisiae*) are more and more widely used in ruminants with the aim to optimise feed utilisation and rumen functions. The National Agronomical Research Institute, France (INRA) and LALLEMAND have been developing research programs for 10 years in order to study the effects and the modes of action of a specific selected strain of *Saccharomyces cerevisiae* (I -1077) in the rumen (Levucell SC).

When Levucell SC I-1077 was co-incubated with *Streptococcus bovis*, an amylolytic and fermentative bacterial species predominating in the rumen when the diet is rich in rapidly fermentable carbohydrates, lactate production by the bacterial species was significantly reduced (1). In the presence of high-concentrate diets, the accumulation of

lactate is generally linked to ruminal acidosis, characterized by a sharp decrease in pH and poor rumen efficiency due to changes of the microbial balance. *In vitro*, Levucell SC I-1077 has demonstrated its capacity to compete with *S.bovis* for the utilization of sugars; the reduction in quantity of fermentable sugars available for the bacteria consequently limited the amount of lactate produced. This effect was only observed when SC was used alive, dead SC I-1077 had no effect on lactate production. Moreover, growth and metabolism of lactate-utilizing bacteria, such as *Megasphaera elsdenii* and *Selenomonas ruminantium* were stimulated in the presence of Levucell SC I-1077 (1). Levucell SC I-1077 could supply growth factors (amino acids, vitamins, organic acids) essential for the lactate-fermenting bacteria. In fistulated sheep receiving Levucell SC I-1077 daily during their adaptation period to a high barley diet, ruminal lactate concentration was significantly lower compared to control animals. Consequently, rumen pH was maintained at values compatible with an efficient rumen function, as shown by higher fibrolytic activities in the rumen of sheep supplemented with Levucell SC I-1077 (2).

In other studies using gnotoxenic lambs harbouring only three species of bacteria as sole cellulolytic organisms, establishment of cellulolytic bacteria took place earlier in the rumen of lambs which received Levucell SC I-1077. During the experiment the lambs were fitted with a rumen cannula, that induced disruptions of the microbial balance, as suggested by the strong decrease observed in the cellulolytic bacterial counts in control animals. In the presence of Levucell SC I-1077, the cellulolytic microflora was stabilized at a high level. Moreover, rumen fiber degradation was significantly enhanced and most of the polysaccharidase and glycoside-hydrolase activities were increased in the presence of the yeast strain (3). These results demonstrate that Levucell SC I-1077 could stimulate growth and activities of cellulolytic bacteria.

In conventional lambs supplemented with Levucell SC I-1077 shortly after birth, populations of cellulolytic bacteria were established earlier and remained more stable than in control lambs (4). The ciliate protozoa also colonised more rapidly in the rumen in the presence of Levucell SC I-1077. It has been stated that the establishment of ciliate protozoa cannot occur unless bacterial and fungal communities have previously colonised the rumen (5). Therefore these results confirm that the maturation of the microbial ecosystem could be accelerated in the presence of the yeast. In current animal production systems, separation of the young ruminants from their mother occurs very soon after birth. The transition from milk to solid feed happens before the end of the sequence of microbial colonisation of the rumen, when the ecosystem is not stabilized yet, which may lead to digestive disorders in the young animal. Daily utilization of Levucell SC I-1077 may therefore avoid these troubles.

It has been observed in lamb studies, previously cited, that ruminal ammonia concentration was lower in the presence of Levucell SC I-1077, suggesting that an improvement of nitrogen retention by the animal could be obtained by modifications of nitrogen microbial metabolism in the rumen. Current research is particularly focused on this aspect of nitrogen retention which has economical as well as environmental implications. Moreover, the stimulatory role of Levucell SC I-1077 on hydrogen utilization by acetate-producing bacteria has been highlighted. *In vitro*, Levucell SC I-1077 increased H₂ utilization by acetogens to the detriment of methanogenic Archaea (6). Experiments are currently undergoing to determine more about the capacities of Levucell SC I-1077 in this field.

Production Responses to Yeast Additives

The cow's response to a yeast additive is a direct result of its action in the rumen. Research on yeast additives can be frustrating because of the often small and variable responses. Better predictability data are needed. Users of yeast additives should also understand the differences between products, knowing that responses may not be similar among them. Understanding the mode of action of a yeast additive more fully will help in predicting action on farms. Also, greater understanding of the impact of diet on cow response to a yeast additive should help.

Yeast Cultures Cow Research

Researchers at the University of California (7) found that cows (n=6) fed yeast culture from 23 days prepartum to 56 days postpartum maintained their normal eating pattern until a time closer to calving (7 days rather than 10 days prepartum) and then regained a normal eating pattern sooner after calving (14 days rather than 20 days postpartum). A larger number of cows (n=44) were used to evaluate intake and performance. Yeast culture did not reduce the decline in dry matter intake prior to calving. Production after calving was slightly improved by the inclusion of yeast culture in the diet. This response was not supported by evidence of enhanced rumen fermentation. There was a slight improvement in dry matter intake of supplemented cows.

A study was conducted with yeast culture by researchers at the University of Wisconsin in 11 commercial dairies (585 cows) having rolling herd averages ranging from 22,000 to 28,000 pounds per cow (8). A one-group TMR was fed on all farms and days in milk averaged 140 days. Eight of the herds had positive milk production responses and on average, milk per cow increased by almost 2 pounds per day. Milk protein yield was also increased (2.57 vs. 2.51 pounds per day) with yeast culture feeding. Milk fat content was reduced from 3.65% to 3.55% but milk fat yield did not change. Using a yeast culture cost of \$0.05/cow/day and a milk price of \$11.82/cwt, the researchers concluded that yeast culture resulted in \$0.13 per day profit per cow. The breakeven response to yeast culture was one-half pound of milk per cow per day. In their study, 73% of the cows achieved at least a breakeven response to yeast culture.

A field study was conducted by Penn State researchers using two *Saccharomyces cerevisiae* yeast products having 10^8 cfu/g live yeast cells (9). There were 306 Holstein cows in their first 120 days of lactation from 7 herds used in the 14-week study. Yeast was top-dressed to supply 5×10^{10} cfu/cow/day. Milk production and milk component production were not affected by treatment although there was a trend for a response in a set of early lactation cows in the study.

A 1999 Penn State study (10) found that feeding 20 g/day of a live yeast + enzyme product (5×10^9 cfu/gram) fed both before and after calving had no effect on intake or milk production during 13 weeks postpartum. Their conclusion was that predicting conditions for a favorable response to live yeast products is difficult. A similar study was conducted at Rutgers University in 1998 (11) using the same product. Here, yeast + enzyme supplementation improved intake (2.6 pounds DM/day), milk production (8 pounds/day), and ADF digestion (2.4%).

Kung, Jr. et al. (1997) conducted two experiments with lactating cows supplemented with a live yeast + enzyme product (12). In the first study, cows were in mid-lactation and received 10 g/head/day of the supplement containing 3.5×10^9 cfu of yeast/gram. Cows produced an average of 72.6 lbs of milk per day and there was no response to treatment. In the second experiment, cows had fewer DIM (75 +/- 25) and were producing more milk. Control cows produced 80.1 lbs of 3.5% FCM/day. Cows fed 10 g yeast + enzyme product per day produced significantly more milk (86.5 lbs of 3.5% FCM/day) ($p < 0.07$). Those fed 20 g produced 83.6 lbs of 3.5% FCM/day but this response was not statistically significant. The authors concluded that differences in response may have been due to interactions among yeast, diet, and stage of lactation. Also, because the product was top-dressed to the cows, numbers of yeast in the rumen may have fluctuated during the day.

Putnam et al. (1997) fed a live yeast product containing 1×10^9 cfu of yeast/gram (10 g/head/day) to early lactation cows fed diets containing 16.1 or 18.8% CP which differed primarily in rumen degradable protein (9.1 vs 11.4% of DM) (13). Supplemented cows on the low protein diet responded in fat yield and 4% fat-corrected milk yield probably because of a slight increase in dry matter intake. Yeast had no effect on microbial protein flow.

Lallemand SC I-1077 Cow Research (an active dry yeast)

Lallemand Animal Nutrition has conducted many cow research studies over the past decade using their active dry yeast product containing *Saccharomyces cerevisiae* (SC I-1077). Levucell SC I-1077 contains 20 billion cfu/gram and is fed at a rate of 0.5 gram/head/day. In a recent project, fourteen of these studies were selected for intensive dietary examination. These trials were selected as accurate information was available on forage and feed analysis, intake and performance. The dietary components and production responses were summarized into a spreadsheet and then the entire database was statistically analyzed. There were 193 observations analyzed in all.

The goal of the current effort was to determine dietary factors that had the most impact on the cow's productive response to Levucell SC I-1077. Rations were analyzed using the CPM Dairy 3.0 to calculate specific nutrient fractions, including protein fractions (A, B₁, B₂, B₃, C) and fermentable carbohydrate fractions (sugars, starch, soluble fiber, NDF). The studies were conducted in Europe, South America, and North America. Diet variation was significant. Dietary forages ranged from 100% corn silage, to mixes of haycrop silage and corn silage, to pasture based diets. In some trials, minimal concentrate was fed while in other studies cows were fed a significant amount of concentrate having a potential negative impact on rumen pH. A broad range in amount and type of soluble fiber was also fed in the studies. Different nutritionists were responsible for designing dietary treatments contributing to variation among studies. The averages, with minimums and maximums from the 14 studies, are presented in Table 1. These data represent the dry matter intake, milk production responses and component responses recorded in the studies. The nutrient fraction concentrations are from the inputs of the rations into CPM Dairy 3.0.

The cows significantly responded, on average, with 3 lbs of milk for the 14 studies. There was also a small increase in pounds of milk true protein and milk fat from the addition of Levucell SC I-1077. For those studies that measured intake, the average response to Levucell SC I-1077 was 1.17 pounds (0.53 kg) dry matter per day. The ranges in milk yield in the studies, were from a marginal response to over 7 lbs of milk and similar changes in milk protein and milk fat, both in pounds and percent. It is this variation that prompted the need to understand more of the factors affecting response to a rumen modifying product such as Levucell SC I-1077.

The data from the spreadsheet were loaded into JMP Discovery software, a statistical product of SAS, for further analyses. The goal, again, as stated above was to examine the interactions of the different fractions relative to the response of the cows to Levucell SC I-1077 so that recommendations could be made in the field for ration formulation practices to enhance the response from Levucell SC I-1077. To that end, stepwise multiple regression was used to establish beginning models to predict milk yield and milk composition. These models were then used to explore the interactions of various dietary parameters using the standard least squares and Effects Screening modules platform within JMP, to examine the interactions. The basic research and the data analysis support the beginning recommendations in Table 2.

Results and Discussion

In the modeling effort with JMP, milk response was pushed to 100 lbs with a higher milk response for the Levucell SC I-1077 cows. The positive milk volume and component response to Levucell SC I-1077 is sensitive to the ruminal ammonia and to the peptides available as a source of branch chained acids required by the fiber bacteria. So, in the recommendations in Table 2, we are suggesting that it is important to enhance the ammonia and peptides in the rumen degradable protein (RDP). NRC (2001) recommends an RDP of a little over 10% of the ration dry matter. Dr. Will Hoover at West Virginia University recommends over 11% DM as RDP with half of that being soluble protein (SIP). The recommendation derived from the Levucell SC I-1077 research fits Dr. Hoover's recommendation. This translates into having the ammonia and the peptide balance at least 110 to 115% of that

required in CPM Dairy 3.0. Additional data analysis suggests that the ammonia and the peptide balance would be more optimum nearer 130% of requirement. This needs to be further investigated.

With the right balance of the degradable protein fractions, there will be a reduction in the risk of acidosis. This will result in a higher contribution of metabolizable energy to the cow and a higher rumen pH resulting in a situation where milk volume and milk components can be enhanced. Dr. James B. Russell, Cornell University, discussed the concept of uncoupled and coupled fermentation years ago. Uncoupled fermentation occurs when there is a deficit of a nutrient or nutrients that would allow the microbes to grow. Fermentation acids (VFA) are the waste products of microbial metabolism. Bacteria will ferment carbohydrates without appreciable growth when fermentation is uncoupled. If we measure the VFA produced and the microbial growth, we can divide the VFA produced by the microbial protein produced. A reduction in this ratio indicates that more of the digested carbohydrate is going into cell mass and not to waste. This is a more coupled fermentation. Alternatively, a higher ratio will indicate a greater production of fermentation acids with little microbial growth, leading to lower rumen pH and disturbance of the microbial ecology, leading to ruminal acidosis. The fermentor data from the West Virginia Fermentation laboratory, the studies from INRA and the lactation studies would suggest that the addition of live yeast will promote a higher degree of coupling and higher fermentation efficiency. The ammonia and peptides are critical to the successful use of Levucell SC I-1077. Interestingly, as rumen pH decreases the proteins from feeds such as soy and canola are less degradable in the rumen. Our first thought is this would be positive. However, this can lead to a further shortage of peptides and ammonia in the rumen leading to further uncoupling and ruminal inefficiency. There is a real need to do further studies in this area.

Interestingly enough, there is the potential for reduced nitrogen wastage with a higher efficiency in the use of dietary protein by the cow. This can happen in two ways. First, because limitations are removed from microbial growth, more rumen ammonia is used, leading to a reduction in loss of this nitrogen from the rumen. Second, with the increase in microbial flow to the small intestine, the quality of the protein absorbed will be improved. This will lead to a higher efficiency of the utilization of the metabolizable protein, reducing urinary nitrogen output, a potential for reducing urea cost and increasing milk protein output. The model called for a marginal increase in RUP, however, practically, we would not formulate for an increased RUP. In reality, if there is an increase in microbial efficiency, there would be a decrease in required RUP. CPM Dairy 3.0 allows us to make a change in microbial efficiency, but we need research to develop the basis for making these types of changes. It should be pointed out that the B₃ pool is decreased, which mostly escapes fermentation. This means that the B₂ pool needs to be a little slower in its degradation in the rumen. There is some small evidence that this might occur when Levucell SC I-1077 is being used.

The fermentable carbohydrate recommendations are higher for the Levucell SC I-1077 addition. The data suggest that the better responses were obtained when this was so. The interaction with the use of Levucell SC I-1077 was modest but definitely suggested an additive response, thus the modestly higher total fermentable carbohydrate (9% increase in fermentable NFC and 4% increase in fermentable fiber), with the starch remaining about the same but fiber, soluble fiber and sugar increasing. These recommendations are tentative, but in formulating rations, it is suggested that close attention needs to be paid to these carbohydrate fractions. There might be concern about the lower peNDF recommended, but the opportunity is there to increase the fermentable carbohydrate, without the fear of ruminal acidosis, and benefit from an increase in microbial protein.

Conclusion and Perspectives

For the last decade we have put too little emphasis in our ration formulation on understanding the dynamics of the rumen and its impact on the productivity of the cow. Basic and good applied research in ruminal metabolism is now allowing us to focus on the opportunities in enhancing productive efficiency at lower costs by enhancing ruminal efficiency. The research with Levucell SC I-1077 is an excellent example of the opportunities in improved ration formulation and also for the need to increase our research in ruminal metabolism. The opportunities are great.

Table 1. Production and Diet Characteristics of Fourteen Levucell SC I-1077 Trials

	Mean	Minimum	Maximum
Milk (lbs)	67.3	33.5	92.8
Milkfat (%)	3.69	2.76	4.52
Milk Crude Protein (%)	3.25	2.90	3.73
Milk True Protein (%)	3.02	2.70	3.47
Dry Matter Intake (lbs)	41.8	24.2	58.5
NEI (Mcal/lb)	0.79	0.68	0.85
Crude Protein (%DM)	17.2	13.9	22.2
Rumen Undegraded Protein (%CP)	33.1	24.3	44.7
Soluble Protein (%CP)	33.4	20.9	47.6
A (%DM)	4.27	0.66	7.75
B ₁ (%DM)	1.4	0.69	3.72
B ₂ (%DM)	8.99	4.84	12.39
B ₃ (%DM)	1.60	0.52	3.56
C (%DM)	0.92	0.57	1.37
Ether Extract (%DM)	4.7	2.9	6.6
Long-Chain Fatty Acids (%DM)	3.7	2.2	5.8
NDF (%DM)	34.4	26.8	42.4
Fermentable NDF (%DM)	13.2	5.7	23.2
Forage (%DM)	53.7	36.4	87.8
NFC (%DM)	39.4	27.6	48.0
Sugar (%DM)	4.8	2.8	10.8
Starch (%DM)	24.6	3.4	36.5
Fermentable Starch (%DM)	20.3	2.9	27.8
Soluble Fiber (%DM)	6.6	2.0	13.4
Fermentable Soluble Fiber (%DM)	5.7	1.7	12.2

Table 2. Recommended Nutrient Concentrations with and without Levucell SC I-1077

Variable	Suggested Concentrations, with no Levucell SC I-1077	Suggested Concentrations with Levucell SC I-1077
Levucell SC 20	-	0.5 gram (10 x 10 ⁹ cfu)
A, %DM	2.9	4.3
B ₁ , %DM	1.8	1.4
B ₂ , %DM	8.9	9.0
B ₃ , %DM	2.1	1.8
C, %DM	0.85	0.9
Total Protein	16.6	17.4
RDP, %DM	11.0	11.5
RUP, %DM	5.6	5.9
Met, %MP	1.96	2.01
Lys, %MP	6.63	6.63
LCFA, %DM	3.7	3.7
peNDF, %DM	26	24.6
Lignin, %DM	3.4	3.4
Fermentable NDF, %DM	12.9	13.4
Sugar, %DM	4.35	6.0
Fermentable Starch, %DM	21.4	21.8
Fermentable Sol. Fiber, %DM	5.4	6.2
Fermentable NFC, %DM	31.2	34.0

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