

Saccharomyces cerevisiae and Aspergillus oryzae, Stimulators and Modifiers of Ruminal Fermentation and Rumen Microbial Growth

Review article

Sahirys Casas Rodríguez

Department of Agronomy, Faculty of Agricultural Sciences, Ignacio Agramonte Loynaz University of Camagüey, Cuba

sahirys.casas@reduc.edu.cu

ABSTRACT

Traditionally, cattle raising has been considered one of the most important sectors of agriculture as a major source of animal protein for human consumption. Accordingly, researchers in different parts of the world have worked for decades on new supplements that modulate ruminal fermentation, increase pH, and ensure the growth of cellulolytic bacteria to enhance fiber digestion and increase milk yields. The aim of this review article is to analyze the role of *Saccharomyces cerevisiae* and *Aspergillus oryzae*, as stimulators and modifiers of ruminal fermentation and rumen microbial growth. Previous research has shown that the best response to additive use in ruminants has been found in dairy cows. The effects observed have been attributed to increased ruminal cellulolysis and the flow of microbial protein into the gut.

Key words: *Saccharomyces cerevisiae*, *Aspergillus oryzae*, microbial growth, ruminal fermentation

INTRODUCTION

Traditionally, cattle raising has been considered one of the most important sectors of agriculture. Ruminants, including ovines and caprines, are some of the major sources of animal protein for human consumption (Fernández, 2012).

Accordingly, FAO (2015) published that the world production of beef in 2008 was approximately 57 million tons, a third of the average annual values. By 2010, production was affected by two main factors: increased production and exports of Western European countries to South America, Australia, and Canada; and The effects of biofuel production on the prices of feedstuffs derived from grains and concentrates (Zicarelli *et al.*, 2016).

In Europe, particularly Spain, cattle represents 5.8% of all agricultural productions. It is the third important economic item, with 15.35% of cattle productions, behind the swine and dairy sectors. Within Europe, it ranks fifth in terms of quality, according to the statistics of 2012. However, a considerable increase (15-30%) in beef production has been predicted worldwide. This significant increase mainly owes to the growing demand

from Southeast Asian countries, particularly (Nájera, 2016).

Ruminants can digest fibrous foods and use non-protein nitrogen sources, creating a tightly close relationship (symbiosis) among ruminal microorganisms to facilitate assimilation (Fernández, 2012).

Accordingly, researchers in different parts of the world have worked for decades on new supplements that can modulate ruminal fermentation, increase pH, and ensure the growth of cellulolytic bacteria to enhance fiber digestion. The most efficient alternative today is the use of probiotics.

Yeast *Saccharomyces cerevisiae* (*S. cerevisiae*) is one of the most commonly used probiotic supplements for the nutrition of lactating cows to improve fermentation in the rumen and therefore, higher milk yields. It was recommended for use in the last decade to stabilize and increase ruminal pH, thus modifying the concentration of volatile fatty acids (Alkhalaf, Alhaj and Al-homidan, 2010; Zicarelli *et al.*, 2016).

Accordingly, Fernández (2012) said also that the utilization of food additives, like *S. cerevisiae*, is very important for ruminant nutrition. However, he stressed the variability of results, with few

repetitions, possibly due to the wide range of diets, different yeast strains, and the amounts supplied to the bovines studied.

Marrero *et al.* (2010), in turn, studied yeast form of fungus *Saccharomyces cerevisiae* together with filamentous fungus *Aspergillus oryzae* (second most important nutritional fungus for ruminants) in lactating cows. It demonstrated that these probiotics did behave as stimulators of microbial growth.

The aim of this review article was to analyze the role of *Saccharomyces cerevisiae* and *Aspergillus oryzae*, as stimulators and modifiers of ruminal fermentation and rumen microbial growth.

DEVELOPMENT

Ruminants

Ruminants are distinguished from monogastric animals by the existence of three compartments: rumen, reticulum, and omasum, arranged before the true stomach or abomasum. These forestomachs have an aglandular mucosa (epithelium that cannot produce digestive juices), which facilitates food degradation, using fiber and non-protein nitrogen. The last of the fourth compartments (abomasum) also has a glandular structure equivalent to the single VIENTRE in mono gastric animals (Redondo, 2003).

Rumen is the largest compartment, and the most important, metabolically. It is an aglandular organ, which does not secrete digestive enzymes. The rumen has one of the most powerful and complex enzyme sets found in nature, which is provided by microorganisms, such as bacteria, fungi, and protozoa, involved in degrading and fermentation processes that take place in the rumen (DePeters and George, 2014).

The rumen is the first compartment where the food goes when ruminants eat. It works as a temporary reservoir and catalyzing chamber in which cellulose is degraded. This process is also known as pregastric fermentation, as it occurs in the anterior portion of the digestive tract, contrary to the intestinal fermentors (Fernández, 2012).

A ruminant nutrition strategy is based on the symbiosis created among the ruminal microorganisms and the animal. The raw materials or other supplies are provided by bovines, provided that the optimum internal environmental conditions, such as, temperature, acidity, anaerobiosis, reductive environment, etc. take place. Meanwhile, bac-

teria make partial use of nutrients and focus on forages, which are not digested by mammals. An also important role is played by fermentation products with nutritional value for cattle, like volatile fatty acids (VFA), and microbial proteins (Sosa *et al.*, 2010).

However, when the relationship is altered by changes in the diet or undesired factors, the unbalance created in the microbiota leads to the occurrence of pathologies (Marrero *et al.*, 2010).

In the rumen, the food ingested by the animal ferments. It is an aglandular organ, which does not secrete digestive enzymes; hence, the gastric activity depends on the enzymes produced by bacteria, protozoa, and ruminal fungi. The relevance of such organisms in the rumen can be explained by the fact that every 15 kg of dry matter consumed by cattle, 10 kg are degraded and fermented by ruminal microorganisms (Sosa *et al.*, 2010).

According to Marrero *et al.* (2010), some of the main functions of microorganisms in the rumen are,

- Digestion of carbohydrates in plants, like cellulose, hemicellulose, starch, and sugars (glucose).
- Conversion of cellulose into short chain fatty acids (SCFA), especially acetic, propionic, and butyric acids (VFA).
- Digestion of proteins from foods.
- Synthesis of bacterial protein.
- Synthesis of vitamins (hydrosoluble, mainly the B and K complexes).
- Digestion of fats.
- Hydrogenation of unsaturated fats.

Rumen as a complex fermentation system

Comparatively, the rumen is a huge fermentation chamber that ensures proper conditions of life and reproduction to the microorganisms that inhabit it. It is the largest reservoir of the digestive system of ruminants, accounting for 70-75% of the gastrointestinal tract, and 50-60% of its volume (Redondo, 2003).

The rumen resembles a continuous culturing system, into which water and food are constantly supplied to microorganisms, along with permanent removal of wastes and final products, whose accumulation might halt the fermentation process. The rumen guarantees stable temperature (39-40 °C), osmolarity, redox potential, and pH to microorganisms (Miron, Ben-Ghedalia, Morrison,

2001). The pH is adjusted between 5.5 and 7.2, partly due to absorption through the ruminal wall of VFA, and the effects of buffers (under physiological rumen conditions it is mainly bicarbonates).

According to Pérez (2000), the microbial population is the largest, representing about 50% of the total microbial biomass. Of the 60 varieties described in the rumen, only 30 or 40 species can be considered autochthonous, whereas the rest has a transient occurrence when foods are contaminated. The gut microbiota made of protozoa (mostly ciliated) comprises 40% of the microbial biomass.

The anaerobic cellulolytic fungi were the latest population discovered and studied. They are particularly abundant in gross forage conditions, but the relevance of their function was not thoroughly investigated. According to the current study, they represent 8% of the microbial biomass.

The essential function of the rumen microbiota, where cellulolytic bacterial species are irreplaceable, is the capacity to degrade and ferment the polysaccharides of the vegetable walls into compounds that can be more easily assimilated by the host ruminant. Its presence and activity depends on a broad set of microorganisms that many times make up true trophic chains (Chiba, 2007).

Main features of microbial cultures

The microbial cultures are made of a mixture of microorganisms (fungi, bacteria, and protozoa), enzymes, vitamins, culture media, and related unidentified factors with beneficial effects during ruminal fermentation. The effect of yeast culture on ruminant nutrition is not stable due to the combination of several components (nucleotides, amino acids, and vitamins), which are supplied to the rumen microbiota through lysis (Pérez, 2000; Cotelo, 2010; Olmedo *et al.*, 2015).

That research coincided with previous studies Hubert (1987), who demonstrated that the microbial cultures have important features:

- They are not pathogenic or toxic.
- They are not absorbed in the digestive tract.
- They leave no traces in animal tissues.
- They are used in small amounts.
- They proliferate *in vivo* and *in vitro*.
- They stimulate the growth of cellulolytic bacteria.
- They are stable at high temperatures.

- They are genetically stable.

Data obtained by Carro and Ranilla (2002) proved that microbial additives, particularly fungi (yeasts and molds), stimulate changes in the rumen microbial population, as well as in their fermentation patterns. It causes an increase in propionic acid, a decrease in methane and lactic acid productions in the rumen, and a decrease in protein degradation and deamination of amino acids. Economic and environmental advantages are produced, as these changes increase the metabolic efficiency of energy and nitrogen in the rumen and the animals (Arcos, López, Bernabé and Hoffman, 2007).

Probiotics

Probiotics were first used in the 1970s by Parker (1974), who coined the term during studies in cattle, saying these microorganisms were able to grow other microorganisms. Fuller (1989) changed that definition, adding that it was a food supplement, which improved gut balance. He also noted that the action mechanism might be competition over receptors in the gastrointestinal tract, and nutrients. He added that it stimulated the secretion of antibacterial substances as well as immunity.

Several other definitions have been suggested since, being the most accepted the one provided by FAO and WHO "... live microorganisms which are beneficial to the health of the host when supplied in proper amounts" (Suárez, Garrido y Guevara, 2016).

Fungi

a) Yeasts

Yeast is a generic name that comprises a variety of single-cell organisms, including plant and animal pathogenic species, harmless species, and useful species (González *et al.*, 2008). *Saccharomyces cerevisiae* is a yeast form of fungus, the group of microorganisms most closely linked to human progress and wellbeing. Its name comes from *Saccharo* (sugar), *myces* (fungus), and *cerevisiae* (beer).

S. cerevisiae is probably the most widely used microorganism throughout history, though in the beginning knowledge was little about its implications in food manufacturing, like beverages and bread (Alvarado, 2011).

Its probiotic effects have been studied both in polygastric and monogastric species (birds and pigs, mainly). Hence, in the last decades, *S.*

cerevisiae has become an important tool for large-scale analysis. This single-cell organism can be easily cultured, and cell division takes place quickly (approximately 2h) (Arcos *et al.*, 2007; Sosa *et al.*, 2010; Fernández, 2012; Zicarelli *et al.*, 2016). (Fig. 1)

S. cerevisiae studies in recent years have demonstrated that not all its strains have the same action mechanism in every animal production system. The differences in response and interaction observed in the cattle diet create new opportunities and challenges in defining the kinds of modifications they cause to ruminal metabolism, depending on the strain type effects and the amounts supplied (Aquilina *et al.* 2014).

Saccharomyces cerevisiae increases food consumption, milk production, feed conversion, and daily weight gain, in response to increases in the quantity and activity of total and cellulolytic anaerobic bacteria that alter the concentration of volatile fatty acids (VFA), ruminal pH, and ammoniacal nitrogen (Pierce *et al.*, 2013).

Action mechanisms of *S. cerevisiae* to increase rumen digestibility

Dawson (1987) said that possibly, the action mechanisms of yeasts that raise digestibility levels are associated to the following:

1. Changes in the bacterial flora by competition and growth stimulation, due to increased cellulolysis and altered microbial synthesis.
2. Modulation of ruminal environment, thus preventing pH fluctuations.
3. Reduction of methanogenic bacterial activity.
4. Optimization of mineral absorption.
5. Supplying of nutrients and essential products like amino acids, vitamins and enzymes.
6. Increase of metabolites as volatile fatty acids caused by greater bacterial activity.
7. Decrease in ammoniacal nitrogen contents.
8. Modification of the amino acid composition in the duodenal flow.
9. Increase of supernatant protein contents.
10. Improvement in voluntary food intake by animals.
11. Reduction of lactic acid concentration.
12. Increase of fiber degradability, according to Olmedo *et al.* (2015) in a recent study.

Effects of *S. cerevisiae* addition on ruminal fermentation.

The enzymatic hydrolyzate of *S. cerevisiae* has demonstrated an activating effect in total viable and cellulolytic bacterial populations in the rumen *in vitro*. Similar results were reported by Marrero *et al.* (2010) who evaluated viable yeasts, and noted that the populations of total viable bacteria increased when yeast strains were used as additives in the diet of ruminants. It coincided with the reports of Sosa *et al.* (2010), who studied the effects of conidial fungus *Aspergillus oryzae* in the diet of ruminants containing fiber.

These results may be explained by the fact that yeasts provide ruminal bacteria with several growth factors, like the B complex, short chain fatty acids (SCFA), branched-chain fatty acids (BCFA), provitamins, and micronutrients (Baiomy, 2011).

S. cerevisiae can duplicate the degradation of the fibrous fraction in the rumen, since it has the enzymatic capacity to penetrate fibrous foods through stomas, colonize highly lignified tissues of tropical forages, and make the lignified parts of the plant cell walls soluble. Its cellulases are considered more active in degrading crystalline cellulase (Sosa *et al.*, 2010; Zicarelli *et al.*, 2016).

Research done on small ruminants by Zicarelli *et al.* (2016) and Pulido (2015) revealed that it was difficult to evaluate the impact of each of these mechanisms on the final effect of yeasts and their cultures. The most probable cause was that the products commercially available included strains that manifested one of these action mechanisms most.

Fernández (2012) and Zicarelli *et al.* (2016), reported differences in ruminal modifications of bovines and ovines-caprines when the animals were fed twice a day, which demonstrated that the dose and frequency of yeast administration may affect the inclusion response.

Moreover, Baiomy (2011) corroborated in his experiments that yeasts increased the flow of microbial protein in the large intestine, but this excess protein would only be beneficial when the diet were deficient.

Marrero *et al.* (2010) added fractions of *Saccharomyces cerevisiae* culture to star grass and evaluated its effect on ruminal fermentation. The results were satisfying, concerning the increase of

ruminal bacteria populations necessary for 30% volatile fatty acid production.

The yeast cultures contain variable portions of live and dead cells of *S. cerevisiae*, which depending on the number of living or metabolically active cells can cause different responses to animal nutrition (Bayat *et al.*, 2015).

Elghandour *et al.* (2016) suggested that the effect of yeast addition in the diet of pre-ruminant calves has promising results. Yeasts positively modify the function of the intestinal tract by speeding the establishment of ruminal and intestinal microorganisms, and preventing the appearance of enteropathogens.

Delgado, Barreto and Vázquez (2015) evaluated the introduction of *Saccharomyces cerevisiae* as a nutritional additive in Siboney calves (180 days old), in Cuba. The study demonstrated the probiotic effects on reducing the incidence of diarrhea and its duration.

Zicarelli *et al.* (2016) found no differences in the fatty acids of milk after adding *S. cerevisiae* in the diet of dairy goats; however, Yalcin *et al.* (2011) reported a significant increase of linoleic/alpha, coinciding with reports made by Bayat *et al.* (2015).

b) Molds

Aspergillus oryzae (AO) is a filamentous fungus known for its probiotic effect. It is characterized by,

1. Increasing fiber degradability, with ensuing raised VFA concentration.
2. Stimulating the growth of bacteria that use lactic acid, such as *Selenomonas ruminantium*.
3. Reducing lactic acid concentration in the rumen, increasing the pH.
4. Increasing the number of cellulolytic bacteria.
5. Improving fiber degradation.
6. Being a source for the manufacture of fermented foods and biomass production (Cortés, Guadarrama, and Díaz, 2014).

Action mechanisms of *Aspergillus oryzae* to increase rumen digestibility

The main action mechanism seems to be related to the capacity of stimulating fiber degradability in the rumen through direct action of fibrolytic fungus *Neocalimastix frontalis*. It also stimulates the production of ammoniacal nitrogen, and in-

creases protein degradability, thus suggesting that *A. oryzae* stimulates proteolysis by contributing with specific nutrients for this kind of bacteria, the presence of proteolytic enzymes in the extract, or access improvements to proteins as soon as the cell walls have been digested (Fernández, 2014).

Ruminal fungi have a high cellulolytic activity, and their function during fiber digestion is probably strategic, opening degradation paths through the cell walls. It allows the access of other bacteria in charge of most quantitative digestion of the fiber (Moallem *et al.*, 2009).

The application of additives has been studied several times with pH and temperature changes, with varying results. González *et al.* (2008) used *Trichoderma* and *Aspergillus* sp., and checked fibrolytic expression at 45 °C, with pH=5.

Moallem *et al.* (2009) observed a high lactose percent of lactose in the milk of cows fed with live yeast, but its combination with the strains of yeast *Aspergillus oryzae* yeast did not produce significant results.

Higginbotham *et al.* (2004), when evaluating the probiotic effects of *Saccharomyces cerevisiae* and *Aspergillus oryzae* separately or combined, in sheep, did not report any differences in milk protein and fat, except for the observation of lactose reduction.

Similar results were achieved by Zicarelli *et al.* (2016), when the diets supplemented with *Saccharomyces cerevisiae* or combined with *Aspergillus oryzae* in sheep, were observed to have no beneficial effects on milk yields. Besides, its composition was worse in the two groups treated, compared to the control. However, it was positive in lactating cows, increasing ruminal cellulolysis and the flow of microbial protein into the intestine, which coincided with reports made by Fernández (2012) and Coteló (2010). They noted that the use of live yeasts and fungal extracts was varied and rarely repeated, possibly due to different diet compositions, different types of yeast strains, and the amounts supplied to the animals in the experiment.

The main issue regarding dosage was the amount of microorganisms in relation to the autochthonous flora to produce a beneficial response in the host, either by administering significant quantities of the microorganisms or by normal growing in the digestive tract (Corcionivoschi *et al.*, 2010).

CONCLUSIONS

Saccharomyces cerevisiae and *Aspergillus oryzae* had both a stimulating and a modifying effect on fermentation and growth of ruminal microbial population.

The best response observed in ruminants was found in dairy cows. The effects observed have been attributed to increased ruminal cellulolysis and the flow of microbial protein into the intestine.

The differences in the results may be explained by the wide diversity of diets administered to the animals studied.

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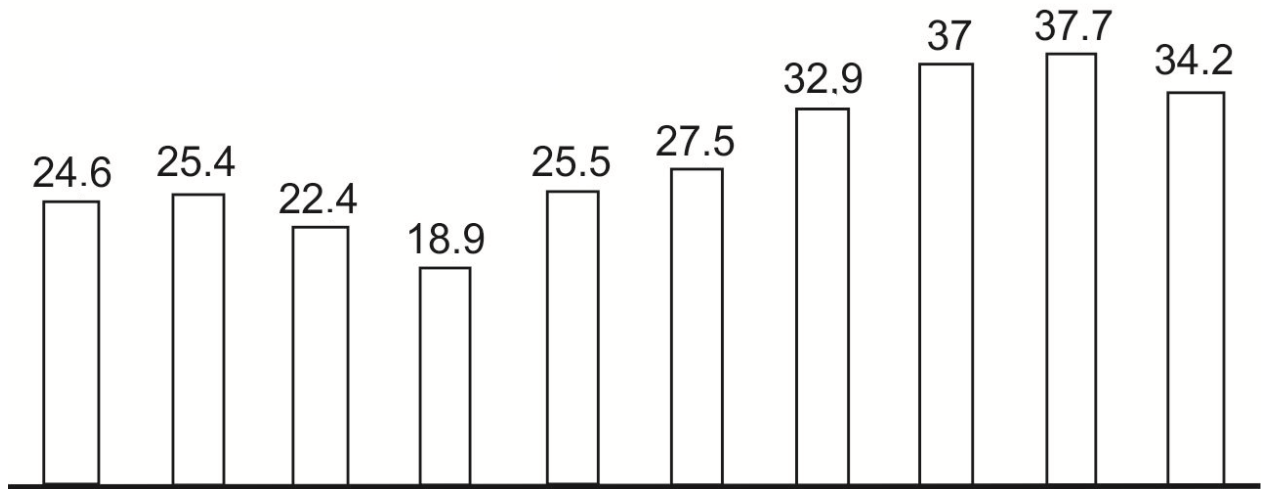
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Production of *S. cerevisiae*



Source: Department of Derivatives, Azcuba

(Fig. 1.) Production of *S. cerevisiae* for animal feed in the last 10 years