Manipulation of ruminal fermentation

I. Introduction

In the last 25 years new feeding strategies have been developed in order to accommodate the ruminal ecosystem to the consumer´s demands, which are based on the improvement of both the productivity and the quality of animal products under a nutritional, sensorial and even therapeutic point of view. In this regard, a number of bio-technological processes have been applied to ruminant animals nutrition aimed to modify, either directly or indirectly, specific metabolic pathways in the ruminal fermentation.

The main interests for this manipulation are:

i) To increase the efficiency of nutrient utilization,
ii) To reduce the risk of metabolic-related diseases and welfare disorders in the stock associated to high-concentrated diets administration (such as ruminal acidosis),
iii) To diminish methane emission: methane excretion implies an energy loss of approximately 3-15% of the energy intake (Van Nevel and Demeyer. 1988). It is also estimated that methane contributes to approx. 15-20% of global greenhouse gases emissions (Cicerone and Oremland, 1988).
iv) To promote the synthesis of certain metabolites whose presence in meat and milk are deemed to exert positive biological effects in the consumer (PUFA´s and CLA).

II. Methods to manipulate the ruminal fermentation:

1.1 Indirect manipulation:

This strategy is based on the manipulation of the macronutrient composition of the diet, including all the physical, chemical and biological treatments required to reduce the ruminal degradation of the dietary protein and starch or to improve the utilization of dietary structural carbohydrates by the ruminal microorganisms.

1.2 Direct manipulation:

This strategy focuses on the modification of specific metabolic pathways by means of using zootechnical additives (organic acids, enzymes, vegetable extracts, probiotics), defaunation or the introduction of genetically modifies species.

III. **Manipulation of nutrient fermentation in the rumen**

1. **Cellulose fermentation**

Cellulolytic bacteria require NH_3, and small amounts of isoacids, which arise from the deamination of the branched amino acids in dietary plant proteins. The pH optimum for cellulolytic bacteria growth is 6.2 to 6.8, which matches the typical ruminal pH of roughage-fed animal. Methanogenic bacteria have similar optimum, and they require a supply of formate, CO_2 and reducing equivalents (2H) to produce methane.

The mixed population of cellulolytic and methanogenic microbes leads to the production of CO_2, CH_4 and the SCFA. The SCFA derived from the fermentation of cellulose – acetate, propionate, and butyrate – are generally in the ratio 75:15:10, respectively (Husveth. 2011).

Indirect manipulation:

Inclusion of buffers to maintain an optimal pH range in the ruminal liquid that enhance the development of cellulolytic bacteria (i.e sodium bicarbonate, sodium phosphates).

Chemical treatments: Na-OH or NH_3, among other alkaline or acidic products that act on vegetable lignified cell walls (Moloney and Flynn 1992)

Direct manipulation:

Protozoa contribute to degradation of vegetable cell walls but also reduce the protein efficiency of utilization due to the ingestion of bacteria and dietary proteins.

Fibrolytic enzymes: Celulasas and xylanasas (Beauchim et al. 2003).

Aspergillus Oryzae: In vivo studies associate its administration to an increase in NDF, ADF, hemicellulose and protein digestibility together with an increase in DM intake (Gómez-Alarcon et al, 1991). In vitro studies relate this additive to an increase in total and cellulolytic bacteria population (Beharka and Nagaraja 1991).

Saccharomyces cerevisae: It is reported that the inclusion of these increase the number of cellulolytic bacterial (Caja et al. 2003) and increase NDF and protein fermentation (Lila et al, 2004).

2. Fermentation of protein

Dietary proteins are classified as rumen degradable proteins (**RDP**) and rumen undegradable proteins (**RUP**).

Bacterial proteolysis of RDP commences with extracellular protease and peptidase activity to produce peptides and amino-acids that are actively absorbed and subjected to further hydrolysis within the bacterial cell. The end-products are ammonia and SCFA, among them the isoacids isobutyrate and isovalerate, which arise from leucine, isoleucine, and valine.

Indirect manipulation:

Feeding regimens must provide sufficient RDP (true protein plus NPN) to meet the requirements of microbial protein synthesis, which depends on the fermentable carbohydrate availability. However, feeding regimens must ensure that excessive protein breakdown to SCFA and ammonia does not occur, as it leads to the overproduction of ammonia, which will be eliminated through the urine as urea, with the consequent loss of nitrogen and the required energy to convert the ammonia to urea in the liver. On the other hand, when the protein requirements are high, as in lactating dairy cows, it is necessary to supplement the microbial protein arriving into the duodenum with RUP. In this case, the use of protein sources of low degradability may be desirable.

Certain natural proteins (as those in maize) and other processed (protected) proteins escape ruminal degradation but can be hydrolyzed by the gastrointestinal enzymes.

3. Fermentation of starch

The degradation of the α-1 linked starches (amylase and amylopectin) and the simple sugars (e.g., sucrose, maltose) is performed by several species of primary amylolytic bacteria. Unlike the cellulolytic bacteria, the amylolytic bacteria have faster fermentation rates and have a lower optimum pH (5.5 to 6.6). This is due to the higher SCFA concentrations with an increase in the relative proportions of propionate, giving a typical acetate/propionate/butyrate ratio of 70:25:5, respectively (Husvéth. 2011). The increased proportion of propionate, as it produces less reducing equivalents (2H), means that there is not such a need for methane to be formed as a sink for reducing equivalents. In turn, this means that less dietary energy is lost as methane.

Secondary bacteria are required for methane formation (methanogenic bacteria) and for the conversion of the lactic acid and other metabolic acids to propionate (propionate bacteria). Both of these groups of secondary bacteria require an optimum pH of 6.2 to 6.8, which is higher than that required by amylolytic bacteria. Therefore, when concentrate diets rich in fermentable carbohydrates, mainly starch, are used, the rapid accumulation of SCFA, may lead to very low pH values for both kinds of secondary bacteria, and as a result lactic acid may

accumulate resulting in chronic or even acute acidosis, that may have a negative effect on animal productivity and welfare.

Protozoa uses starch granules reducing the amount of fermentable carbohydrates available for bacteria fermentation, thus reducing the risk of acidosis. However, when the pH falls below 5.5 pH, protozoa are quickly inactivated and later die.

Indirect manipulation:

-Increase of particle size, and a combination of starch with low/rapid fermentation rates (corn/barley).
- Buffers to maintain an optimal pH for secondary bacteria.

Direct manipulation:

Modulation of the amylolytic bacteria/secondary bacteria ratio by using zootechnical additives (organic acids, vegetable extracts, probiotics).

-Organic acids:

Fumaric/Malic acid are intermediate metabolic products of the trycarboxylic and the 2-methylcytrate metabolic cycle in which pyruvate is transformed to propionic acid.
These compounds are reported to stimulate lactate utilization by *Selenomas ruminantium*, a common gramm-negative bacterium that account for up to 50% of the total viable count in the rumen of animals fed high level of concentrates (Caldwell and Bryant, 1966), and its conversion to propionate (figure 1). Concretely, L-lactate uptake by S.ruminantium was fourfold and 10-fold increased by 10 Mm of fumarate and by L-malate, respectively (Nisbet and Martin 1990). In addition, in the presence of H+ within the rumen, S. ruminantium is able to ferment both fumaric and malic acids into succinate and then to propionate (figure 2). Thus, administration of these organic acids have been associated to an increase in propionate production, to a decrease in methane production (due to the decreased hydrogen concentration) and to ruminal pH rise in animals fed diets with a high amount of cereal grains (Martin et al. 1999).

Figure 1. Extracted from the review of Husvéth (2011)

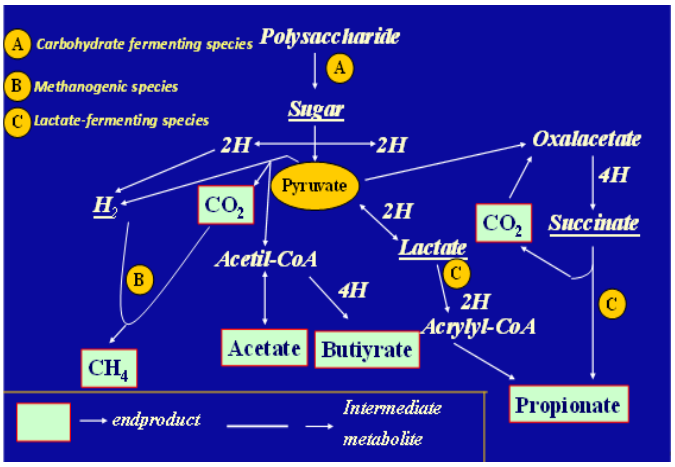
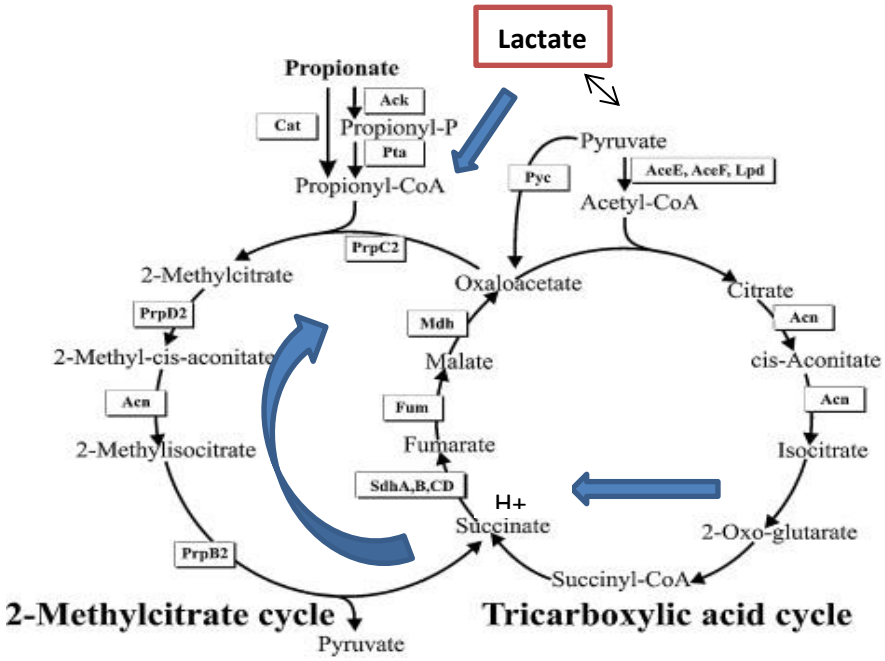


Figure 2. Mechanism of action (arrows) of organic acids (fumarate and malate)



Cons: high dose required: economic cost, and sides effects on palatability and food intake. A possible alternative is to combine low doses of organic acids together with other additives such as vegetable extracts and probiotics (*Caja et al. 2003*).

-**Vegetable extracts**:
Yucca shidigera: (high level of saponins), promote a decrease of Gram positive bacteria and protozoa in the rumen. It has been associated to an increase in microbial protein and SCFA synthesis (Cardozo et al. 2004).

4. Fermentation of dietary lipids

The lipid fraction in leaves of herbs and grasses ranges from 30 to 100 g/kg DM, much of which is contributed by chloroplast lipids (Bauchart et al.1984). Fresh grass contains a high proportion (0.50–0.75) of total FA content as α-linolenic acid. Concentrations of α-linolenic acid are also affected by processes such as plant detachment (i.e. grazing and cutting) and storage. Thus, wilting prior to ensiling reduced the content of total FA by almost 30%, with a reduction of up to 40% in linolenic acid (Dewhurst et al. 1998) and hay making reduced total FA by over 50%, with a higher loss of linolenic acid (Doreau and Poncet, 2000).

Ruminant diets generally do not contain more than 5% DM as lipids. Higher values may have adverse effects on food intake, cellulolytic activity (Jenkins, 1993) and forestomach motility.

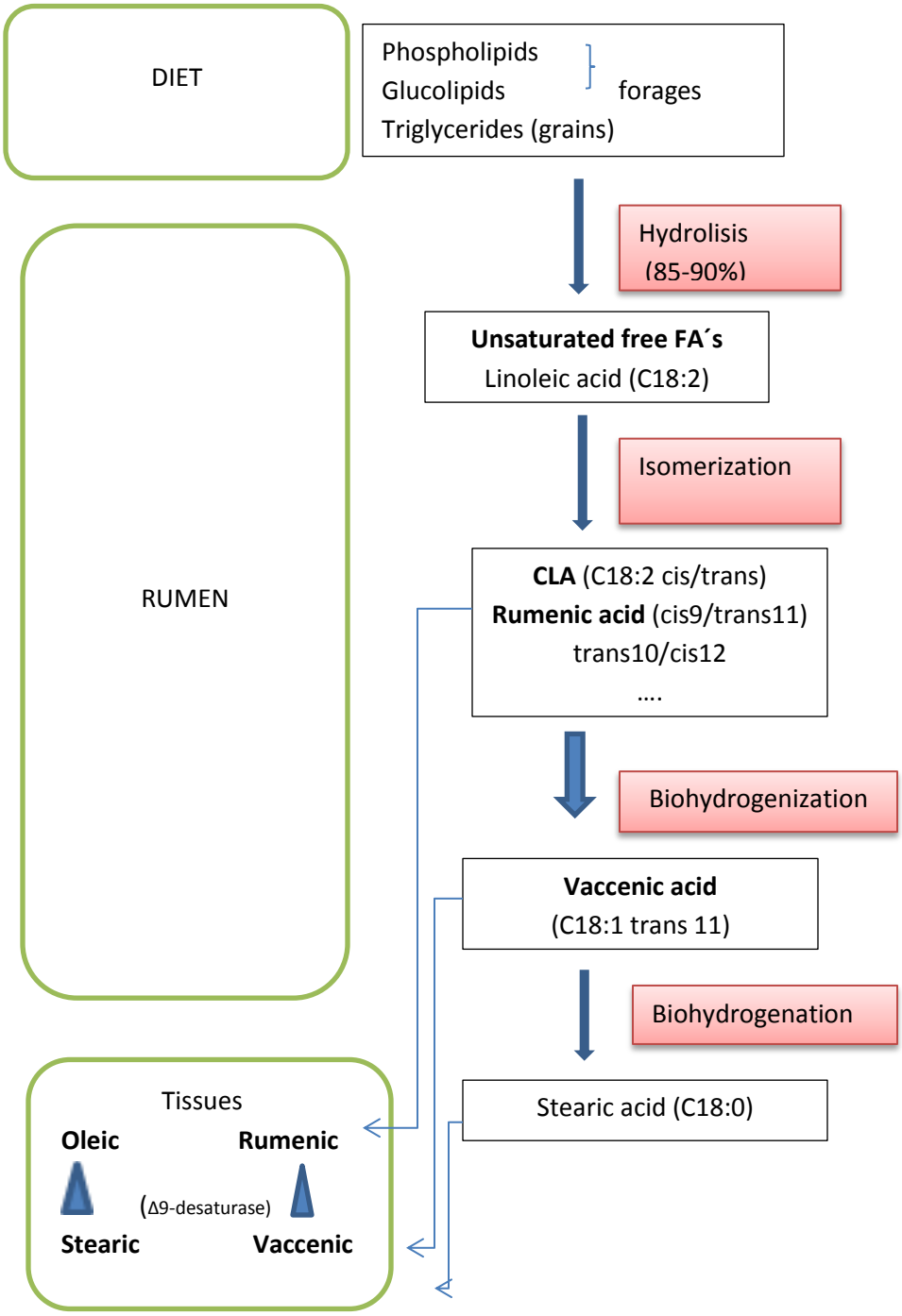
Lipids digestion in the rumen results in the formation of different intermediary metabolites that influence the type and proportions of fatty acids that are finally laid on animal tissues.

Ruminal microbes rapidly hydrolyze dietary lipids and, using the unsaturated fatty acids (oleic, linoleic, linolenic) as hydrogen acceptors, quickly convert most of them, to free saturated fatty acids, namely stearic acids (2/3) and palmitic acids (1/3); Martinez (2007).

Previously, isomerization of 1 cis double bound of linoleic acid (also of linolenic acid) to a trans conformation drives in the formation of different CLA isomers, with rumenic acid (C18:2 cis 9,trans 11) being the predominant one. After absorption, stearic acid and vaccenic acid (C18:1: trans-11) are desaturated by the enzyme Δ9-desaturase and stored as oleic acid and rumenic acid, respectively in body fat and milk. Activity of this enzyme, however, may be reduced in the presence of CLA C18:2 trans10, cis12 (Loor et al., 2003). Figure 3.

Protozoa have an important role in ruminal lipid metabolism. They absorb some of the polyunsaturated fatty acids (PUFA), lock them away in their own structure, and thereby protect them from hydrogenation. The protozoa that subsequently flow out of the rumen and undergo intestinal digestion release their PUFA content, this being probably the main source of PUFA for ruminants (Husvéth.2011).

Figure 3. *Ruminal synthesis of CLA from dietary unsaturated fatty acids*



Reasons for lipid fermentation manipulation:

Interest in CLA food content arised after the discovery of its health-promoting properties, including its potential anticarcinogenic and antiteratogenic effect (Chin et al., 1992; Banni and Martin, 1998).

Rumen lipid fermentation manipulation might be aimed to i) increase CLA content in tissues destined for human consumption as a means to increase CLA intake in human subjects; ii) reduce body fat in the animal and iii) enhance the health of the animal based on its immunomodulatory effects (Azian. 2003).

Milk fatty acids profile comprises 69% Saturated FA´s (SFA´s), 27% mono-unsaturated FA´s and only 0,04% PUFA´s (Jensen et al. 2002). Despite its low PUFA´s content, milk still provides a relevant amount of CLA, in both cis-9, trans 11 and trans-10, cis-12 forms reaching variable concentrations among herds depending on the diet. Ruminant meat is also an important source of CLA (2,9-4 and 5,6 mg/g fat in beef and lamb meat, respectively) in comparison to the meat of monogastrics (0,6 and 0,9 mg/g fat in pig and poultry meat, respectively, with rumenic acid representing more than 85% of the CLA content found in muscular fat (Chin et al., 1992). Concretely, it is considered that endogen desaturation of vaccenic acid to rumenic acid by the Δ9-desaturase is the main origin of this CLA (Piperova et al., 2002).

Nutritional management is primarily directed to increase dairy and meat products CLA content by maximizing rumen output of vaccenic acid (which has a more stable configuration than rumenic acid, and thus has a higher tendency to accumulate in the rumen). This can be achieved in two ways: by increasing the supply of 18-carbon PUFA precursors and by inhibiting vaccenic acid reduction to stearic acid.

These are the main feeding strategies described in the literature to increase CLA content in milk and body fat.

1. Pasture grazing has been shown to promote a significant increase in milk CLA content without affecting milk fat content. In the study of Dhiman et al (1999), cows grazing pasture alone had 500% more CLA in milk fat than cows fed diets containing forage and grain in a 50:50 ratio, although those animals showed a milk yield reduction.

2. Forage to grain ratio exerts also a pivotal role in CLA ruminal biohydrogenation. High-concentrated diets and the associated ruminal pH decline is reported to inhibit ruminal lipolysis and to alter FA´s saturation process, driving in an incomplete biohydrogenation characterized by an increased output of CLA 18:1, trans 10, cis 12, probably due to an altered rumen environment (Latham et al., 1972; Griinary et al., 1998**).** Since CLA C18:2 trans10, cis12 is reported to reduce milk-fat synthesis in the mammary gland (Baungard et al, 2000), low forage

to grain ratio have been deemed to contribute to the milk-fat depression syndrome in dairy cattle. At this respect, Griinary et al (1998) found a depression in milk and fat yield of 30 and 35%, respectively, associated to a higher content in CLA trans 10, associated to low fiber diets administration (ratio forage:grain; 20:80), but only when the diet was supplemented with unsaturated FA´s (UFA) supplement , effect that did not occur when UFA´s supplement was added to high-fiber diets (ratio forage:grain; 50:50). These findings indicate that UFA´s supplementation directed to increase CLA content might be a good recommendation when a minimum amount of effective fibre is provided, so that a balanced ruminal environment. Current NRC feeding guidelines suggests that minimum ration NDF levels for lactating cows should be in the 25-28% range and that 75% of this NDF should be provided by forage.

3. Supplementation with Unsaturated fatty acids (UFA´s)

Oils rich in linoleic acid (soybean oil) have been found to enhance vaccenic acid (C18:1, trans11) and rumenic acid ($C_{18:2}$*cis*-9, *trans*-11) CLA´s content in milk fat than oils containing linolenic acid (flaxseed oil) in dairy cows fed high-forage diets (59% forage) (Bu et al. 2007).

Addition of long chain PUFA´s (EPA, DHA) have demonstrated to be effective in increasing CLA milk concentration (Lee et al .2005). However the prohibition of using animal feedstuff to ruminant animals limits its employment to just experimental conditions. The alternative of using microalgae as PUFA´s source is still economically very costly.

4. Incorporation of protected CLA

Supplementation up to 80 g/d protected CLA rised CLA milk content from 0,80% (control) to 3,97 % milk fatty acids. Higher doses (160 g/d), however had a depressor effect in DMI (Piamphon et al. 2009).

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