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Popular Article

Study of degradation of feeds in ruminants: Basic concept and methodology

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Abstract

Rumen degradation is crucial in the supply of dietary nutrients to meet the nutrient demands of the anaerobic microbes and body tissues of ruminant animals. To assess the nutritional status of ruminant animals, rumen degradation of feeds that they receive must be estimated. Therefore, it is essential to study the dynamics of rumen degradation of various feeds before their potential use to formulate nutritious diets for ruminant animals.

Carbohydrates

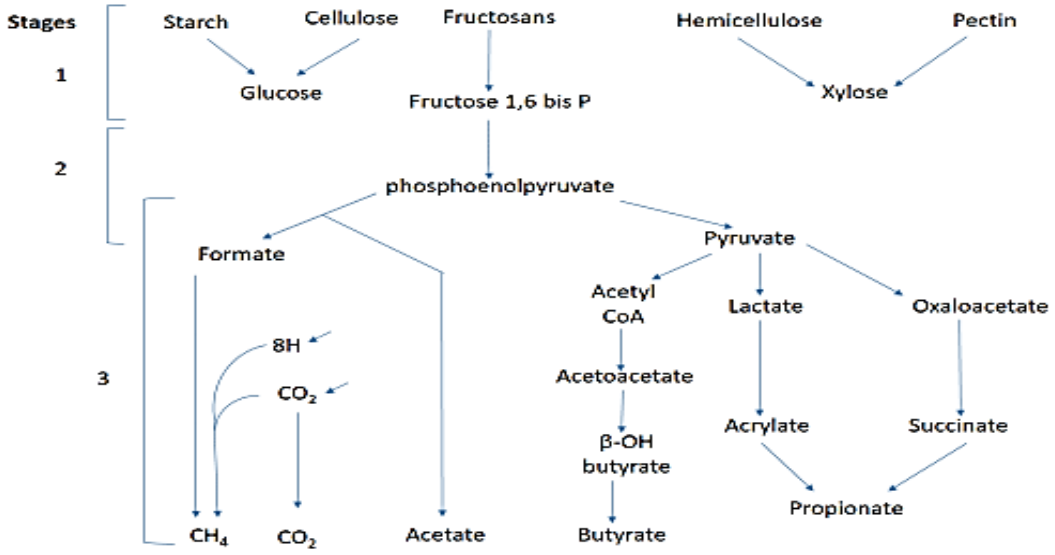
Types of feed consumed by ruminants includes roughages and concentrates. The roughage (grasses) constitutes of structural constituents such as cellulose and hemicellulose. The enzymes (cellulose, hemicellulose, pectin lysase, and fructosanases) necessary to hydrolyze β-1 linkages are found only in microbes and plants. Therefore, herbivores require a symbiotic relationship with suitable microbes (bacteria, fungi and protozoa).

In concentrates (grains), most of the carbohydrates is non-structural and intracellular, present as stored energy (starches and fructosans) or synthesis intermediaries (simple sugars). The α-1, in contrast to the β-1, linkages are readily hydrolysed by enzymes (amylases), which are present in the alimentary tract secretions of most animals as well as on microbes and plants.

Constituent carbohydrate	**Structural unit**
Cellulose	β-1,4-linked glucose units
Hemicellulose	β-1,4- linked xylose units
Pectin	β-1,4-galactouronan
Starch	α-1,4-glucose linkages without side chains (**amylose**)

	α-1,4-glucose linkages with side chains (**amylopectin**)
Fructosans	β-1,2 fructose units

Fermentation pathways



The first three stages of a four-stage microbial process.

Stage 1: Hydrolysis of plant polysaccharides to their constituent monosaccharides and then conversion of these to fructose-1,6-bisphosphate. This is reached via glucose for starch and cellulose, via fructose for fructosans, and via xylose for hemicelluloses and pectin.

Stage 2: Embden-Meyerhof pathway for the anaerobic oxidation of fructose-1,6-bisphosphate to pyruvate via phosphoenolpyruvate.

Stage 3: It covers the reactions that produce the final metabolites of fermentation. Phosphoenolpyruvate is the origin of the pathway leading to the acetate, some butyrate, and a transient intermediate, formate, which is then converted to methane. Pyruvate is the origin of the pathways that

1. via β-OH butyrate produce butyrate
2. via oxaloacetate and succinate ("the randomizing pathway") produce propionate, and
3. via lactate and acrylate ("the direct reductive pathway") produce 10-30% of the total propionate.

Stage 4: Synthesis of microbial compounds, particularly amino acid formation, using stage 1 to stage 3 intermediates coupled with transamination and energy (ATP) derived from the fermentation (Embden-Meyerhof) pathways.

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Fermentation of cellulose

The degradation of the β-1-linked compounds (cellulose, hemicellulose, fructosans, pectin) is performed by several species of primary cellulolytic bacteria, which are capable of the four stages of microbial activity except for methane production (carried out by methanogenic bacteria). The fermentation of cellulose is slow because of low metabolic rate of cellulolytic bacteria (18 hrs to double their numbers).

For protein synthesis, cellulolytic bacteria do not require a supply of amino acids, but need NH_3, the stage 2 and 3 intermediates, and small amounts of isoacids, which arise from the deamination of the branched amino acids in dietary plant proteins. The pH optimum is 6.2-6.8. The methanogenic bacteria require a supply of formate, CO_2, and reducing equivalents (2H) to produce methane and a supply of amino acids to meet their protein requirements. The mixed population of cellulolytic and methanogenic bcateria leads to the production of CO_2, CH_4 and VFAs (acetate: propionate: butyrate in the ratio of 75:15:10).

Fermentation of starch

The degradation of the α-1-linked starches (amylose and amylopectin) and the simple sugars (e.g., sucrose, maltose) is performed by several species of amylolytic bacteria. Some of these are capable of all four stages of microbial process, except for methane formation, whereas others carry out stages 1 and 2 but cease with the production of one of the metabolic acids, most commonly lactic acid.

The amylolytic bacteria have faster fermentation rates, have much shorter doubling times (0.25 to 4 hrs), and have a lower pH optimum of 5.5-6.6. The acetate/propionate/butyrate ratio of 70:25:5, respectively.

Fermentation of dietary protein

Bacterial proteolysis commences with extracellular protease activity to produce peptides that are actively absorbed and subjected to further hydrolysis within the bacterial cell. The end products are amino acids, some of which are taken up by other microbes and the remainder deaminated to produce ammonia and various metabolic acids. These are fermented to VFAs and include small amounts of branched-chain VFAs (the isoacids, isobutyrate and isovalerate), which arise from leucine, isoleucine and valine and are required as minor nutrients by the cellulolytic bacteria.

Fermentation of dietary lipids

Ruminal microbes rapidly hydrolyze dietary lipids and using the unsaturated fatty acids (oleic, linoleic, and linolenic) as hydrogen acceptors, quickly convert most of them to stearic acid. Ruminant diets generally do not contain more than 5 % dry matter (DMA) as lipid. Higher

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values have adverse effects on (1) food palatability, (2) cellulolytic activity, (3) food appetite and forestomach motility, probably as a result of negative feedback by the cholecystokinin released when fat is present in the duodenum, (4) the physical consistency of concentrate pellets at high and low temperatures, and (5) the shelf-life of concentrates, lipids being prone to the development of rancid flavors.

Protozoa have an important role in ruminal lipid metabolism. They absorb some of the PUFAs, lock them away in their own structures, and thereby protect them from hydrogenation. The protozoa that subsequently flow out of the rumen and undergo intestinal digestion release their content of PUFAs, this being probably the main source of PUFAs for the ruminant.

Methods to study degradation of feeds in ruminants

The degradability of various feedstuffs can be determined by *in vivo*, *in sacco/in situ* or *in vitro* methods.

In vivo: The quantity of nutrients flowing to the duodenum or abomasum of fistulated animals is measured to determine feed degradability.

The in vivo method is unsuitable for routine feed evaluation because it is laborious, expensive, requires large quantities of feed and is largely inappropriate for single feedstuffs. This method also subjects to errors associated with the use of digesta flow-rate markers, microbial markers and inherent animal variations. These methods are unreliable due to the large variation observed among animals as because only a few animals can be used in in vivo experiments because of increasing concern for animal welfare.

In vivo methods involving internal and external markers

The protocols require animals fitted with cannulae in the reticulo-rumen, the abomasum, or proximal duodenum. Also they require suitable methods for determining digesta flow rates and for differentiating microbial protein from dietary protein in the digesta that flows to the small intestine. Therefore, several microbial markers have to be used which may be classified as internal markers that are inherently present in micro-organisms and include diaminopimelic acid, aminoethylphosphonic acid and nucleic acids (DNA or RNA) or external markers (that are added to the rumen to label the micro-organisms) including 35S, 15N, 14C, 3H and 32P, etc. Despite the use of several markers, there is no single ideal marker to estimate the ruminal microbial protein yield.

In sacco or in situ: The in sacco method was first suggested by Quin et al. (1938). It has been concluded that as long as the bags were large enough to allow free movement of substrate within, the technique could be extremely useful as a rapid guide to determine nutrient disappearance, particularly the rate and extent of nutrient disappearance from the rumen. All

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modern systems of feeding ruminants require an estimation of the amount of feed protein escaping ruminal degradation. This estimation is obtained by the in sacco technique, which is probably the best-known simple and reliable method to assess the degradability of DM and protein in the rumen.

The in sacco method involves the sealing of feed samples within nylon, polyester or Dacron bags suspended in the rumen of fistulated animals for varying periods of time followed by estimation of degradability of feedstuffs from the measurements of DM or N disappearance in the washed residues and particle flow rate from synthetic porous bags containing test feeds. However, the reproducibility among laboratories for this method is poor partly due to the variation in proteolytic activity between animals due to their variable diets and physiological status, etc. Therefore, the results obtained for this method may not be equally applicable to all situations unless the method is standardised for a common protocol. Besides possibility of microbial contamination within the bag, other possible sources of variations in the use of the in sacco method exist among different laboratories in terms of bag size, sample size, particle size and time (hrs) of incubation used by different authors.

The in sacco method has been the most effective method to study rumen degradation amongst many methods that have been used in the past. However, it requires surgically prepared animals so, it is undesirable due to its implications for animal welfare and costs. It is relatively less labour intensive and so is cheaper as compared with the in vivo method.

In vitro methods: These methods involve buffers, chemical solvents, rumen fluid and enzymes that are either commercially available or extracted from rumen contents. Another approach is to use gas production as an indirect measure of in vitro digestion. In vitro techniques are considered less expensive than the in vivo and in sacco methods, and they offer the possibility of analysing both the residue and the metabolites of microbial degradation.

In vitro methods may ultimately allow for the control of various factors that alter the feed degradation (microbial, animal, environment) and, therefore, allow for the uniform characterisation of feeds for DM and protein degradation.

Tilley & Terry developed an in vitro method to estimate the apparent DM digestibility of feeds for ruminants in the laboratory. The method has two stages. In the first stage, a feed sample is incubated at 38°C in rumen fluid, which is diluted with a buffer solution resembling saliva and saturated with carbon dioxide. After 48 h, the incubation is stopped and the incubation mixture filtered. The feed residues are subsequently incubated for another 48 h with pepsin-HCl. The main disadvantage of the method is that rumen fluid is required, which is obtained from fistulated animals, and may not be available in all laboratories.

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Difficulties associated with the in vitro fermentation studies

- Requirements to standardise the fermentation process
- Measurement of fermentation profiles
- Access to fistulated ruminants to obtain rumen inocula

Therefore, several methods have been developed to measure nutrient degradation by using solvents and buffers and enzymes preparations.

In vitro methods involving solubility in solvents and buffers

Several researchers have attempted to characterise feed nutrients according to their solubility in aqueous solutions such as saline and buffers, autoclaved rumen fluid (ARF) and water (cold, hot or distilled). However, most of the available reported literature on feed solubility focuses on protein solubility. Protein solubility is influenced by various factors associated with the solvent or extraction procedure. Changes in chemical composition of solvent can have pronounced effects on N solubility. It is difficult to draw meaningful conclusions as to the effect of solvent composition on N solubilization, because of the interaction that may exist among feedstuffs, methodologies and solvents.

The pH of a solvent has been shown to influence N solubility of concentrate feed protein. However, the effect of pH on protein solubility often is measured in neutral pH solutions, even though rumen fluid is slightly acidic when donor animals consume high-grain-based diets. As sodium chloride has almost no buffering capacity and bicarbonate – phosphate buffer has an unstable pH, a borate – phosphate buffer has been suggested as an appropriate substitute to measure N solubility without variation due to fluctuation in pH.

In vitro methods using enzymes

Various enzyme preparations involving cellulases, proteases, lipases and amylases individually or as mixtures have been used. Ruminal protease enriched from *Bacteroides amylophilus* and *S. griseus* have been used for studies of degradation of several protein sources. Indeed, the procedures involving the use of commercial proteases offer potential advantages over other techniques, particularly in terms of the labour and speed of operation.

A drawback in relying on the proteolytic activity of just one specific bacterium, as opposed to a group of microorganisms, is that the whole range of activities towards the different nitrogenous substrates found in the rumen may not be present in a single enzyme. Not all species of bacteria can degrade and utilise nitrogenous substrate to the same extent. This suggests that the use of a single enzyme, even if extracted from a rumen microorganism, may not be appropriate for an accurate estimation of the total proteolytic activity of the whole rumen fluid.

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In vitro methods involving gas production

Gas produced has been reported to be primarily from the fermentation of digestible carbohydrates like monosaccharides, polysaccharides, pectin, starch, cellulose and hemicellulose in the form of CO_2 and CH_4 by the activity of rumen microbes. The procedure used for gas collection and measurement ranges from the use of calibrated syringes and pressure transducers.

The advantage of the gas production systems is that they can be automated, thus reducing the labour input. However, automated gas production methods are expensive and may not handle large numbers of samples.

In vitro method involving faeces

It is well established that certain amounts of cellulose and hemicellulose are fermented in the large intestine, because many bacterial species in the rumen are also represented in the hind gut from where bacterial residues are subsequently passed in the faeces. Therefore, the suspension of faeces in buffer might be capable of acting as an inoculum for the initial fermentation of feed samples, in place of rumen liquor. Certain experiments suggest that in vitro digestibility determined by using faeces as an inoculum correlated well with the in vivo digestibility.

Conclusion

Feeds when ingested by ruminant animals are subjected to microbial degradation in the rumen. The information on rumen degradation of different feeds before their use to formulate nutritious diets for ruminant animals is essential. However, it would be essential to standardise the protocols for each method (*in sacco* and alternatives). This standardisation should aim to minimise the potential sources of variations among different laboratories that estimate degradation of feeds being used to formulate nutritious diets for ruminants.

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