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In vitro gas methods for evaluation of feeds containing phytochemicals

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Abstract

Measurement of gas, a reflection of short chain fatty acids (SCFA), by in vitro gas methods provides information on effects of phytochemicals on rumen fermentation. However, to obtain complete information, it is necessary to measure other end products of fermentation, in particular microbial mass, especially for studies involving phytochemicals or bioactive moieties. Examples using tannins, saponins and alkaloids are discussed, highlighting the limited and often misleading information that can be obtained on potential effects of these compounds by measuring only gas production. In these studies, microbial protein was determined using purines or diaminopimelic acid as a marker, or by ^{15}N incorporation into microbes. In addition, the suitability of using the difference between apparently and truly degraded residues, as the estimate of microbial mass, for evaluation of tannin-free fibrous feeds, low-starch feeds, or when the objective is to investigate potential effects of phytochemicals other than tannins, is discussed. However for tannin-containing feeds, the presence of tannin–protein complexes in these residues, and/or solubilization of tannins from the substrate that do not contribute gas or microbial mass, produce artifacts in apparently and truly degraded values, rendering this approach invalid. For quantification of truly degraded substrate in tannin-rich samples, an indirect method based on level, and molar proportion, of SCFA and microbial mass is suggested. An approach for microbial mass determination, especially useful for tannin-rich samples, based on N balance is also discussed. An advantage of gas methods is that the fate of a phytochemical in the rumen can be investigated simultaneous with its effect on rumen fermentation. Such studies, using condensed tannins, alkaloids and saponins are presented. The in vitro gas method is a relatively simple and inexpensive tool to study potential effects, mechanisms of action and fates of phytochemicals in the rumen. The method

Abbreviations: SCFA, short chain fatty acids; ATP, adenosine triphosphate; DAPA, diaminopimelic acid; PEG, polyethylene glycol; DM, dry matter; NDF, neutral detergent fibre; NDIN, neutral detergent insoluble N; IVDN, in vitro degradability of N; RNA, ribonucleic acid

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could also be used to screen for novel bioactive moieties such as those having antimethanogenic, antiproteolytic and antiprotozoal activity.

Keywords: Phytochemicals; Plant secondary metabolites; Tannins; Saponins; Bioactive compounds; Microbial protein; Gas method

1. Introduction

In vitro methods to determine nutritional quality of feeds are important to nutritionists. These methods are less expensive, less time consuming and allow more control of experimental conditions than in vivo experiments. A number of gas measurement techniques and in vitro gas methods have been used by several groups to evaluate the nutritional value of feedstuffs (Getachew et al., 1998a). The in vitro gas method based on syringes (Menke et al., 1979) appears to be most suitable for use in developing countries where resources may be limited (Makkar, 2004), and it has been established in over 30 countries in the last 5 years through projects sponsored by FAO/IAEA. Increased interest in use of non-conventional feed resources has led to an increase in use of this technique, since gas production can provide useful data on digestion kinetics of both the soluble and insoluble fractions of feedstuffs. The ease of measuring fermentation end products makes this method more efficient than other in vitro methods for studies on phytochemicals, plant secondary metabolites and feed additives. For example, efficiency of microbial protein synthesis, and mechanisms of action of phytochemicals and other bioactive moieties including feed additives, can be studied with relatively inexpensive equipment, and in less time, since a large number of samples can be handled at one time. In addition, in vitro gas methods allow better monitoring of nutrient–phytochemical and phytochemical–phytochemical interactions.

This paper reviews results of phytochemical–rumen microbe interactions, including principles and approaches that may be applied in studies of effects of other bioactive compounds, natural or synthetic, on rumen fermentation.

2. Importance of measuring microbial mass with gas measurement in studies on phytochemicals or other bioactive moieties

The need to determine microbial mass while measuring gas production has been highlighted for evaluation of feed resources, particularly forages (Blümmel et al., 1997; Getachew et al., 1998a; Makkar, 2004). In this context, it is important to note that in vitro gas measurement reflects only short chain fatty acid (SCFA) production, and the relationship between SCFA and microbial mass production is not constant, probably because of variation of biomass production per unit ATP generated (Blümmel et al., 1997; Getachew et al., 1998a).

In my laboratory, incubation of 0.6 mg/ml of various saponins in a gas method affected gas and microbial mass production to different extents (Table 1). For example, addition of