In-Vitro Gas Production Technique

Subjects: Zoology

Contributor: Zahia Amanzougarene , Manuel Fondevila

The in vitro gas production technique, either based on volume or pressure measurements, was initially set up for the evaluation of the rate and extent of fermentation of feeds for ruminants. Since it is carried out under pH conditions simulating a well-bu ered medium (from pH 6.5 to 6.8), it has been generally focused to evaluation of forages and fibrous by-products, or by estimating fermentation of concentrate feeds (cereals, protein sources) for extrapolation of their use in mixed diets. However, it has also been used for determination of the nutritive value of feeds in all-concentrate diets, without taking into account that in such cases pH may range between 6.5 and 5.8, and often below this range, creating unfavourable conditions for bacterial fermentation. Modifying the concentration of bicarbonate ion in the incubation solution allows to adjust the incubation pH to conditions that simulate the in vitro fermentation conditions to those occurring under high-concentrate feeding. This highlights the importance of the incubation pH for the estimation of fermentation of feeds.

gas production pH bicarbonate ion high concentrate feeding semicontinuous system.

1. The Gas Production Technique

The *in vitro* gas production technique, either based on volume or pressure measurements, was initially set up for evaluation of the rate and extent of fermentation of feeds for ruminants. Gas systems are generally adapted to forage feeding conditions, with pH ranging in a range close to neutrality (between 6.5 and 7.0). However, it has also been used for determination of the nutritive value of feeds in all-concentrate diets, without having into account that in such cases pH may rather range between 6.5 and 5.8, and often below this range, creating unfavourable conditions for bacterial fermentation.

2. Factors influence PH

Under ruminal conditions, the contribution of bicarbonate is responsible for the maximum buffering capacity. Because of this, incubation solution for the different versions of the procedure include buffering solutions of different composition to maintain pH within levels mentioned above. In most cases, such solutions are made up mainly with bicarbonate buffer plus a minor proportion of phosphate buffer, where the contribution of phosphate buffer at the incubation range of pH can be considered as residual. Several attempts using different buffers have been made to control incubation pH in order to evaluate microbial fermentation under conditions simulating high concentrate feeding, but results are not completely successful because of rapid exhaustion of buffering capacity. Recently, a modification of bicarbonate ion concentration in the buffer of incubation solution has been proposed, reducing it concentration from 110 mM to levels established according to the desired incubation pH.

Another factor of major concern is the source of inoculum. The composition and type of diet given to the donor animals determines its fermentative capacity and the degree it fits to the substrate to be studied. Thus, rate and extent of fermentation of a fibrous or a concentrate feed highly depends on its evaluation with inoculum from an animal fed on either a forage- or a concentrate-type diet. The incubation pH can also be affected by the type of inoculum.

The adjustment of buffer concentration, together with using rumen inoculum from donor ruminants given high-concentrate diets, allows for mimicking concentrates fermentative conditions *in vitro*. It is important to consider that the gas volume recorded is in part directly produced from microbial fermentation of substrates, but also indirectly from the buffering capacity of the medium. Thus, the contribution of each (direct and indirect) gas source to the overall production should be estimated, and the determination of the volatile fatty acids concentration may help for doing it.

A major factor affecting fermentation is the rate of passage, but closed batch systems cannot be adapted to its consideration. Therefore, a simple semicontinuous incubation system has been developed, which estimates the rate and extent of fermentation by the volume of gas produced at a given time, but also allows for controlling medium pH and rate of passage by manual replacement of incubation medium by fresh saliva without including rumen inoculum. Application of this system to studies under high concentrate feeding conditions allows for the in vitro study of the effect of high concentrate diets and highlights the importance of the incubation pH for the estimation of fermentation of feeds.

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