Chapter 5

Factors Affecting Rumen Fermentation Using Batch Culture Technique

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Additional information is available at the end of the chapter

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Abstract

The method of batch culture has been widely applied to evaluate feed value and screen feed additives. The advantages of using this in vitro technique as compared to in vivo methods are many, including low cost, simplicity, requirement of small quantities of feed or additives and the ability to screen large numbers of samples under similar experimental conditions. However, the number of factors associated with the batch culture could alter fermentation outcomes. This chapter discusses the potential impact of series factors on in vitro fermentation and the considerations on improving application of batch culture in ruminant nutrition. The factors that are discussed include inoculum source, gas-recording methods, substrate particle size, substrate delivery method, ratio of rumen inoculum to buffer in mixture of media and addition of soluble carbohydrate in media. Some recent important results obtained using batch culture technique have been highlighted and discussed. Any particular batch system being accepted as the 'standard' procedure seems difficult. However, before any protocol can be adopted, sufficient data need to be developed to reduce the variation and improve the consistence of the measurements.

Keywords: batch culture, feed evaluation, gas production, inoculums, rumen fermentation

1. Introduction

Rumen fermentation plays a major role in feed digestion and microbial production in ruminants. The rate and extent of feed digestion in the rumen, rumen fermentation pattern and amount of microbial protein production ultimately determine the feed value, nutrient provision and animal productivity. Therefore, determining the feed digestibility in the rumen is necessary to predict

animal production and optimum ration formulation. In addition, substantial feed additives are presently used to improve or modify rumen fermentation and their activities need to be determined. The use of animal to measure either feed digestibility or activity of feed additives is a reliable approach but disadvantages are numerous such as time consuming, expensive, require large quantities of feed (or feed additives), and unsuitable for large-scale feed evaluation. As a result, many biological methods which simulate the rumen fermentation process have been developed.

The method of batch culture has been widely applied to screen and compare various feeds and feed additives (e.g., feed enzymes). The advantages of using in vitro techniques as compared to in vivo methods using animals include low cost, its simplicity, small feedstuff requirement and particularly the ability to screen large numbers of samples under similar experimental conditions [1]. However, a number of factors used in the batch culture method including inoculum source, recording system of gas production, method of substrate dispersal in the bottle, sample size and method of substrate preparation could alter fermentation results [2–4]. For example, venting methods for gas measurement is a noticeable issue. In a closed system, gas accumulates and the rise in pressure in headspace may affect the rate of substrate fermentation [5]. Different venting systems to relieve gas pressure have been compared, but results on feed digestion have been inconclusive [2, 6]. Tagliapietra et al. [3] reported that using manual pressure measurements, headspace volume, venting frequency and amount of fermentable substrate need to be carefully balanced to avoid high headspace pressures that could alter fermentation kinetics. Other researchers have reported placing substrates in porous bags within incubation vials [1, 7] or placing it freely into the inoculum [8, 9]. Greater amounts of methane were observed from samples directly dispersed in vials as compared to that enclosed in bags [4]. It is possible that the bags create a microenvironment that is distinct from that of free inoculum and may vary with changes in the pore size of bags [10]. The substrates that are incubated in batch culture need to be processed to obtain an adequate particle size prior to incubation because of lack of mastication and rumen contraction occurs in animal. The use of a finely ground sample reduces the risk of sampling bias, especially for forage samples, but fine particles may exit the bags prior to true digestion. All these factors related to batch culture have not been standardized across the laboratory, and they could significantly impact the fermentation results, thus increase the variability and reduce the reliability of the method. The objective of this chapter is to discuss several key factors that potentially influence the outcomes of the batch culture and to provide useful information to better use the batch culture technique in the evaluation of feeds or feed additives in ruminant nutrition.

2. What is the batch culture?

Batch culture is a technique for large-scale production of microbes or microbial products in which, at a given time, the fermenter is stopped and the culture is worked up. The 'batch culture' fermentation is also known as 'closed culture' system. In this system, at the beginning, the nutrients and other additives are added in required amounts. There is no refill of nutrients once the fermentation process has started and the product is recovered at the end of the process.

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In the beginning, microorganisms grow at a rapid rate due to availability of excess nutrients. As time passes, they increase in number with rapid use of the nutrients and simultaneously produce toxic metabolites. The batch culture that is currently used to evaluate ruminant feeds or feed additives is primarily based on the in vitro technique developed by Tilley and Terry [11] and modified by Goering and Van Soest [12]. The batch culture consists of collection of rumen fluid as inoculum, inoculation of dried, ground feed samples contained in a flask with a buffering and nutritive in vitro medium. Sample digestion is measured following anaerobic degradation by rumen bacteria. The batch culture can measure the kinetics and volume of gas production (mainly CO_2, CH_4), as well as gas profiles, rate, and extent of substrate digestion, which can then be used to evaluate feed values (ranking feed) and feed additive screening. The kinetics of gas production or feed digestion can be a developed model to predict feed intake, microbial protein synthesis, and metabolizable energy.

During the fermentation of feedstuff, the truly digested substrate is partitioned among volatile fatty acids, gas and microbial biomass. Gas production occurs when substrate carbohydrates are fermented to generate acetate or butyrate but no gas is produced with fermentation of carbohydrate to generate propionate. However, gas is also produced when volatile fatty acid causes gas to be released from the bicarbonate buffer [13]. Although gas production is a reflection of the generation of volatile fatty acids and microbial mass as a result of substrate fermentation, gas measurements only account for substrate that is used for volatile fatty acids and gas production and does not consider substrate utilized for microbial growth. Therefore, the volume of gas produced during fermentation is highly associated with the amount of substrate digested. Currently, the gas production technique is commonly used to evaluate and predict feed value and screening feed additives for ruminants. One major advantage of in vitro gas measurement technique is that it focuses on the appearance of fermentation products and non-fermentable substrates do not contribute to gas production [13]. Since gas production does not consider the amount of substrate converted into microbial biomass, the substrate digestibility that is estimated based on gas measurement is considered as apparent digestibility [14]. Feed protein degradation does not contribute to gas but the high ammonia nitrogen concentration in in vitro systems might prevent the release of gas due to its highly basic nature. As one of major measures of batch culture, gas measurement is widely used to predict rate and extent of feed digestion in the rumen as well as feed intake and microbial protein synthesis [14].

3. Factors affecting batch culture fermentation

3.1. Effect of inoculum source

The inoculum is often the major source of variation on the variable measurements in the use of batch culture technique to study fermentation kinetics of ruminant feeds. The effect of inoculum source on in vitro gas production was considerably discussed in a review by Rymer et al. [2]. Considerable animal variation in the quality of rumen fluid inoculum, prepared identically, is known to exist both within and among donor animals [15, 16]. The variation of batch fermentation due to inoculum source is ultimately attributed to the variation of microbial

population profiles and microbial activities in the rumen. Therefore, all the factors that potentially affect the ruminal microbial activity would affect inoculum quality, thus varying the batch fermentation. In this section, the effects on batch fermentation of the inoculum from sampling schedule, different species, rumen versus faeces as well as inoculum preparation are discussed.

3.1.1. Effect of donor animals, diet and collection time

The donor animals, type of diet and the inoculum collection time may all have an effect on consistency of fermentation results between cultures. It is well known that there is considerable individual animal variation on rumen pH and rumen fermentation pattern under the same feeding and management conditions. Therefore, it is often recommended to collect rumen inoculum from several animals and then combined to reduce the variation. Recently, we have conducted a batch culture to compare rumen inoculum of cattle with low- and high-feed digestion. It was observed the differences in gas production and dry matter digestibility of barley straw when the low- and high-feed-digesting rumen inocula were used. However, the use of such inoculum did not result overall in the differences in gas production kinetics. The effect of the inoculum sources on the in vitro effective dry matter digestibility agrees with previous reports that a difference in the activity of the inoculum exist among individual donor animals [16]. In another batch culture using always the same inoculum (low- versus high-feed-digesting cattle), we observed that the gas production and dry matter digestibility of barley straw werenot affected by inoculum source. The results suggest that inoculum from high-feed-digesting cattle did not necessary improve in vitro digestion of straw.

The rate and extent of feed degradability in the rumen vary with the type of feeds and feed processing. Therefore, diet is considered as a significant factor influencing the inoculum activity. Cone et al. [17] reported that the degradability of starch from different feed sources was greater for the donor cow fed a diet containing equal concentrate and hay compared with a hay-based diet. However, the composition of the concentrate mixture had only a minor effect on degradability values. It is clear that the ruminal microbial activity was different between cows fed hay versus hay and concentrate mixed diet. However, manipulating concentrate composition would not dramatically change ruminal microbial profiles. Mertens et al. [18] reported that the higher-fibre diets tended to produce more gas than the lower-fibre diets, which may explain by more acetate production with high-fibre diet, since fermentation of substrate fibre generates primarily acetate and gas is produced when substrate is fermented to generate acetate or butyrate rather than propionate. Huntington et al. [19] showed a similar response when dry cows were fed a diet of either straw or grass silage with rolled barley, and no differences in the gas production with a diet of a dried grass. Menke and Steingass [20] indicated that there was little difference in gas production of treated straw when hay in the diet of donor animals was replaced with treated straw. The inconsistent effect of donor animal diet suggests that it is more important to ensure the minimum microbial activity in the rumen fluid, rather than ensuring that donor animals are fed the substrate incubated.

Rumen microbial activity is increasing following feed ingestion, thus different sampling times have been applied to collect inoculum in literature either for obtaining high activity (i.e., 2 h

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after feeding) or for reducing variation (i.e., before feeding). Cone et al. [21] reported the increased rate of fermentation with rumen fluid that was collected after the morning although the total gas production was not affected. Menke and Steingass [20] stated that sampling rumen contents just before feeding reduced variation in activity of the inoculum. Although differences in microbial activity of inoculum occur at different sampling times, it appears that the most important factor is whether the sampling schedule will allow collection of inoculum with sufficient microbial activity. Payne et al. [22] observed less variation between replicates when rumen fluid was collected either 4 or 8 h after feeding, compared with before or 2 h after feeding.

The rumen fluid preparation procedure had relatively little effect on gas production [23]. However, Bueno et al. [24] reported an increase of in vitro organic matter digestibility by increasing the proportion of the solid phase relative to liquid phase in inoculum preparation and concluded that the contribution of microorganisms from the solid phase of rumen inoculum is important, especially in studies to evaluate high-fibre feeds. Recently, comparing the rumen inocula from low- and high-feed-digesting cattle, we did not find the differences in fibre digestibility of barley straw between the two inoculum sources, which may be explained by the method of inoculum preparation. Although whole ruminal contents were collected, rumen inoculum was obtained by squeezing manually, and it would represent primarily the bacteria associated with liquid or loosely associated with feed particles but not with bacteria tightly associated with particles. The proportion of bacteria associated with rumen feed particulate has been found to range from 50 to 70% and mainly characterized as fibrolytic bacteria [25].

3.1.2. Inoculum from different species

Rumen fluid from sheep is often used as inoculum on the batch culture because housing sheep is easier and less expensive than the cattle, whereas the results obtained with batch culture technique are mainly used to evaluate feeds for beef or dairy cattle. As a result, numbers of studies were conducted in comparison of rumen fluid between cattle and sheep on in vitro gas production and rumen fermentation [24, 26]. Cone et al. [26] compared rumen fluid from cows and sheep fed a similar diet, and they found that the gas production was lower with sheep rumen fluid, but there was a good relationship between volumes of gas produced by the two inocula. They concluded that sheep rumen fluid could replace cow rumen fluid for accurate determination of 24 and 48 h gas production and the gas production profile. However, rumen fluid of cows could not be replaced by that of sheep for the rate of gas production determination. Similarly, Bueno et al. [24] observed the similar gas production and degradability between sheep and cattle under the same feeding and management conditions. However, kinetics of gas production differed between species and so dynamic determinations, such as rate of gas production data, using sheep inoculum cannot be extrapolated to cattle. Bueno et al. [27] found the similar gas production and organic matter degradability of tropic forage between cow and sheep rumen fluid, whereas rumen fluid from sheep resulted in gas production with a longer lag time (6.1 h versus 4.2 h). Differences in microbial composition of rumen fluid from these sheep and cattle appeared to especially affect kinetics of fermentation, but not the end point

measures. Few studies were compared between cattle and buffalo on the effects of rumen fluid on rumen fermentation. Calabrò et al. [28] found higher gas volume and earlier maximum rate of substrate degradation with cow than buffalo inoculum. All of these data indicate that species of donor animal will affect rumen fermentation.

3.1.3. Rumen versus faecal inocula

Use of faecal inoculum in batch culture has been paid great attention in scientific community during last two decades as it would overcome the need for surgically modified animals. The comparison between rumen fluid and faecal inoculum on in vitro gas production and extent of feed fermentation were well documented in several review articles [2, 15]. In general, the use of faecal inoculum give lower cumulative gas production and feed digestibility than use of rumen fluid although a good correlation is often determined. It suggests that the microbial activity in faecal inoculum is lower than in rumen inoculum. The difference between rumen and faecal inoculum may vary with feed degradability in the rumen. When the diet of the donor animal is highly fibrous, such that the microbial activity of the rumen is low, then differences between rumen fluid and faecal inoculum would be smaller, but when high-productive animals are used, the faecal inoculum are of limited value. Mauricio et al. [29] stated that the faecal inoculum could replace rumen fluid where incubations were over extended periods and cumulative gas volumes were examined since the gas release kinetics differed up to 48 h of incubation between the two inocula. Cone et al. [26] concluded that cow rumen fluid cannot be replaced by cow faeces for determination of 24 h gas production, but to be a good alternative for cow rumen fluid to accurately determine 48 h gas production. Mould et al. [15] suggested that faeces may replace rumen fluid as an inoculum for end-point measures (i.e., degradability or cumulative gas volume at the end of extended incubation periods); faecal material is likely an unsuitable inoculum for estimating rate of fermentation.

3.2. Manual versus automated methods

The gas generated from batch fermentation is generally measured either manually using the manual pressure transducer developed by Theodorou et al. [5] or automatically with the automated systems as described by Pell and Schofield [30], Cone et al. [21] and Davies et al. [31]. It has been reported that the headspace gas production associated with feed fermentation can be manually measured by inserting a needle attached to a pressure transducer into the vials at fixed time points [1, 8], or measured automatically using a transducer recording system [32]. Theoretically, the automated recording system, which vents gas at regular intervals may be more accurate than the manual system as where headspace gas can reach higher pressures. Accumulation of gas (i.e., the rise of gas pressure) may influence the release of gas from buffered ruminal fluid [3] and reduce the fermentation rate of substrate [5]. In closed systems, where gas is not released and accumulates, the rise of pressure in the headspace may cause a staircase effect in the recorded data. Especially with fast-fermenting substrates, some of the headspace gas may be forced into the liquid phase, and this dissolved gas may not be released instantly in the following reading, thus affecting successive measurements. Several studies were carried out to compare the gas

produced using manual pressure transducers and automated pressure systems. The studies by Rymer et al. [2] and Gierus et al. [6] have observed greater gas production with the manual procedure than automated system. Similarly, we previously used two gas production systems, which were differed in gas pressure recording (automated versus manual), headspace and sample size of the bottle. Serum bottles (100 mL) sealed with a rubber stopper were used for manual gas pressure recording and a 500-mL Ankom gas production module (a computerized system with automated pressure transducers, Ankom Technology, Macedon, NY, USA) equipped with an Ankom pressure sensor module including a microchip and a radio transponder was used for automated gas pressure recording. The result also showed that the gas production was different when gas pressure was recorded using the two systems but it was interacted with the type of substrate incubated. The gas production was higher using manual system when the substrates had higher digestibility such as alfalfa hay and wheat distiller grains, whereas no difference in gas production was observed with the incubation of barley straw which had lower digestibility. The similar gas production of barley straw between the two systems may reflect the slower digestion rate of straw generating less gas. In addition, the gas production values from manual and automated recording systems in our study were calculated from different formulas, this may have biased gas production estimates. For the manual system, the gas volume was calculated using the equation described by Mauricio et al. [33]: gas volume, mL = 0.18 + (3.697 × gas pressure) + (0.0824 × gas pressure^2), whereas for the automated system, the gas volume was estimated according to Avogadro's law (gas volume, mL = gas pressure × [V/RT] × 22.4 × 1000, where V is headspace volume in the bottle in litres, R is the gas constant 8.314472 L kPa/K/mol, and T is the temperature in Kelvin). Rymer et al. (2005) reported the stronger relationships between laboratories with manual system than with automated system and suggested that the increased complexity and cost of automated system may not be repaid by increased value of the results. However, the automated system produced good reproducibility among laboratories [21].

3.3. Effect of material delivery

The feed substrates can be incubated directly by dispersing in the medium or incubated in a filter bag. Incubating feeds in filter bags has been widely applied in batch culture [1, 8] because of its practical convenience. In comparison with dispersing the substrate into the medium, enclosing feed in bags has the advantage of being able to simultaneously determine in vitro digestibility of dry matter and fibre without the need to capture residues after incubation. However, incubating feeds in bags can have concerns on restricting microbial access to the substrates, particle loss from the bags during incubation, and the accumulation of the fermentation products which may inhibit microbial activity [34]. The lower in vitro dry matter and fibre digestibility was reported when feeds were incubated in filter bags as compared to when feeds were dispersed in the medium [35]. Krizsan et al. [36] suggested that this lower feed digestibility may arise from the inability of microbes to readily gain access to substrates within the bags, thus lowering the digestion. Additionally, the possible poor fluid exchange within the bags may result in an accumulation of the fermentation products which could further inhibit the fermentation. Ramin et al. [4] reported lower methane production for feed incubated

in filter bags than dispersed in medium because of reduced feed digestion in vitro; however, the proportion of methane to total gas production was greater for feeds incubated in bags than for feeds dispersed in the medium. It was suggested an alteration of microbial population or fermentation pattern of the feeds incubated in bags versus feeds dispersed in the medium. There was also interaction between feed and method (i.e., bag versus dispersing) on ranking of methane output. It was concluded that the bag method should not be used when measuring methane emission during 48 h of incubation. In contrast, our recent study [9] showed that the incubation of feeds in filter bags consistently increased the digestibility of dry matter and fibre as compared to when the feeds were dispersed in the medium. The discrepancy with other studies may be resulted from the low densities of feed substrates being incubated. In this study, Barley straw, alfalfa hay and wheat distiller grain were ground through either 1- or 2-mm sieve and were incubated. It was observed that some feed particles were floating on the top of media and adhered to the sides of the bottles as a result of agitation during incubation. Obviously, this portion of the substrate would not come in direct contact with microbial populations and thus feed digestion would be compromised. Additionally, incubating substrate in bags to measure dry matter digestibility may have potentially resulted in overestimation of digestibility due to possible washout of feed particles from bags. The washout fraction varied with substrate and ranked as wheat distiller grain (18.8%) > alfalfa hay (12.1%) > barley straw (5.9%). However, because the washout fraction could primarily depend on the soluble fraction, and the soluble fraction is considered to be highly fermentable in the rumen, the impact of washout fraction would be minimal for the dry matter digestibility at longer incubation hour, for example, 24 h, whereas it would have a significant impact on the gas production kinetic measurement. He et al. [9] suggested that the method of substrate delivery could be a primary factor to be considered if the dry matter digestibility is a key variable measured. Therefore, lower microbial activity within the bags, altering microbial population or fermentation pattern of the samples incubated in bags compared with those directly dispersed in the medium show negative aspects of the bag method, but such disadvantages may have limited impact when using the batch culture for screening feed additives or ranking feedstuff. The practical convenience of using bags is highly attractive, thus commonly used currently.

3.4. Effect of substrate particle size

Feed digestion in the rumen requires that microorganisms colonize and produce enzymes that hydrolyse feed particles. Increasing the feed surface area increases the accessibility of microbes to substrate, thus potentially increasing feed digestibility. Anele et al. [37] reported that more processed barley grain (i.e., lower processing index at 0.75 which is calculated as ratio of density after rolling to the density before rolling) produced more cumulative gas volume than less processed barley samples with processing index of 0.85 due to less fermentable nutrients. Yang et al. [38] also reported higher in vitro gas production and dry matter digestibility of ground barley (1-mm sieve) as compared to dry-rolled barley (processing index at 0.80), suggesting that grinding increased the surface area available for microbial attachment. However, Rymer et al. [2] suggested that with highly soluble feeds such as some cereal grain, as long as the feed has undergone some abrasion, its particle size does not affect estimates of gas production rate. Lowman et al. [39] reported the similar gas production profiles of

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incubated naked oats that were cut at half, quarter, coarse and finely ground except the whole naked oats. Similarly, McAllister et al. [40] found the similar in situ dry matter digestibility of halved and quartered grains but significant lower dry matter digestibility of whole grain. Seed grains are generally protected by the pericarp and a processing by rolling or grinding is necessary to make the nutrient-rich endosperm available to the microbes, and to increase the rate and extent of digestion. However, there are many evidences that particle size of processed grain has significant impact on in vitro digestibility. Rymer et al. [2] indicated that maize grain that was steam-flaked, rolled or left intact had the same rate and extent of gas production when it was ground through a 1-mm screen but not when it had been ground through a 4-mm screen. With fibrous and more slowly degraded feeds, gas production rate increases as particle size decreases [39] and it appears that the increased gas production has resulted from an increased surface area as a result of grinding, thereby allowing better microbial access. It seems that there is interaction between feed particle size and type of feeds on in vitro gas production and dry matter digestibility. In the study of He et al. [9], a greater digestibility of dry matter and fibre of alfalfa hay ground 1 mm over 2 mm was observed; however, the digestibility of dry matter of barley straw did not respond to the particle size, and even less gas production and fibre digestibility with barley straw ground through a 1-mm screen as compared to a 2-mm screen was noticed. There was no clear explanation on this unexpected finding and authors speculated that finer straw adhered to the bottle more readily due to its greater buoyancy resulting in a lower digestibility.

The feeds that are incubated in vitro are often finely ground (e.g., ground through 1-mm sieve) since the particle size reduction during in vitro incubation is minimal due to absence of mastication and ruminal contractions. The use of finely ground sample also reduces the risk of sampling bias considering usually only less 1.0 g of sample is included in incubations. Yang et al. [38] concluded, based on a comparison of 60 barley samples either ground or dry rolled, that grinding is likely an appropriate processing method to evaluate digestion characteristics of barley using batch culture technique. In fact, the starch digestibility of ground barley after 24 h of incubation was similar to in vivo values observed in the rumen [41]. There was also less variability in digestibility and better correlation between chemical composition of barley and in vitro digestibility for the ground than the rolled barley. The advantage of using finely ground sample has no concern on processing quality associated with kernel uniformity. However, one can make the argument that barley that is tested in vitro should be processed in a manner similar to the form that it is fed to the animal. Although this approach does not consider the impact of mastication on digestion, it is equally clear that fine grinding also eliminates any sample-mediated differences in the particle sizes that may be generated after dry rolling of barley. Nocek [42] stated that reducing the variability of particle size by grinding through 1-mm sieve may not mimic the in vivo conditions ideally but it does tend to improve the precision of both in vitro and in situ measures. Yang et al. [38] reported the low correlation for digestibility of dry matter between ground and rolled barley (R^2 = 0.12), and suggested that the processing associated with kernel uniformity affected at least partly the digestibility of rolled barley. It can be concluded that the impact of particle size on in vitro feed digestibility can be significant but vary with the type of feed incubated. If the gas production technique is to be used as a means of feed evaluation, it may be necessary to require a standardized particle size

and sample preparation procedure in order to reduce variation among experiments and laboratories. Adoption of a standardized approach to sample preparation may be possible to enable comparison between independently produced gas production and digestion data of different feeds. Additionally, as substrate particles are continually changing shape, size and composition in the gut, it seems unlikely that gas production or dry matter digestion data will represent kinetics of plant biomass as it is digested in the rumen.

3.5. Ratio of rumen inoculum to buffer

The ratio of rumen inoculum to buffer varies considerably in the various batch culture techniques from 1:9 to 1:4 Cabral Filho et al. [43]. Increasing the proportion of rumen inoculum in the incubation medium reduced lag time of gas production, but increased the volume or the rate of gas production [23, 30]. Navarro-Villa et al. [44] incubated with three different ratios of rumen fluid to buffer (i.e., 1:2, 1:4, and 1:6), and observed the increased gas production per unit of dry matter input, CH_4 to gas production and CH_4 to total volatile fatty acid ratio in all feeds incubated with increasing the proportion of rumen fluid in the mixture. The increase in CH_4 output due to change of rumen inoculum to buffer ratio can be resulted from different fermentation pattern, such as for barley grain appeared to be associated with higher acetate to propionate ratios and for barley straw was due to higher volatile fatty acid production. There was also a quadratic response of dry matter digestibility to increased ratios of rumen fluid to buffer with feed dependent, wherein decreasing the ratio resulted in a decline in digestibility with barley grain, an increase with grass silage and an increase (between 1:2 and 1:4) followed by a larger decrease (between 1:4 and 1:6) with barley straw. The decrease in ratio of rumen fluid to buffer would decrease microbial activity of the mixture media, thereby reduced feed digestibility. Pell and Schofield [30] included rumen fluid at the proportions of 5, 10, 20 and 40% in the total medium mixture, and observed the increase of alfalfa hay digestibility with increasing the proportion of ruminal fluid. It suggested that a 20% inoculum is sufficient to ensure the maximum rate of fibre digestion but lower percentages of inoculum are not sufficient. The increased lag time without altering maximum gas productions by lowering the ratio of rumen fluid to buffer appeared to reflect the time required for the microbial numbers to increase to levels comparable with those in the higher inocula. The microbial activity in rumen fluid can be determined by measuring absorbance of the inoculum following a 50-fold dilution at 600 nm and it is recommended a minimum microbial activity of 94 mg bacterial DM/ml [45].

3.6. Effect of concentrate addition on roughage fermentation

The inclusion of readily digestible carbohydrates in forage-based diets for ruminants can restrict microbial digestion of structural polysaccharides because rumen pH can be below the optimum [46]. The rumen pH below the optimum level is especially unfavourable for microbial fibrolysis. However, when poor quality of roughage such as straw is incubated in batch culture, there may be nutrient deficiency to support microbial growth or lack of fermentable carbohydrate to attract microbes to adhesion on the substrate, consequently reducing digestibility of substrate. Barrios-Urdaneta et al. [47] reported that the low available energy content of the

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straw cell wall that was incubated in vitro resulted in low fibre digestion even after long hours of incubation (i.e., 72 h). In addition, the low energy was also responsible for low numbers of bacteria associated with the substrate and a low level of polysaccharidase activity, both of which were corrected by the inclusion of energy supplements. Several studies indicated that the source of carbohydrate inclusion could also influence in vitro cell wall fermentation of crop straw. The higher in vitro straw cell wall digestion was observed with addition of pectin versus soluble sugars or starch [47] or when supplemented with sugar beet pulp, a source of highly digestible structural carbohydrates, compared with barley grain as a source of starch [46]. Barrios-Urdaneta et al. [47] suggested that the effect on increased cell wall digestion of straw was mainly attributed to higher bacterial adhesion to cell wall particles at early incubation time. We conducted a batch culture to incubate barley straw alone or barley straw plus a concentrate mix. For the treatment of straw + concentrate, 30% of barley straw was replaced by the equal amount of concentrate mix which consisted of 60% corn distillers grain, 22% canola meal, and 18% mineral and vitamin supplement in dry matter basis. The concentrate was incubated in a second bag within serum bottle. We observed greater rate of gas production and a shorter lag time with adding concentrate than the incubation of barley straw alone. An increased soluble fraction and dry matter degradability as well as increased fibre digestibility of straw by adding concentrate were noticed. The concentrate used in our study consisted of primarily corn distillers' grain which contained very low starch, but high protein and fibre. The fibre in corn distillers' grain has twice hemicellulose compared to original corn and it is highly fermentable in the rumen. Additionally, the protein from concentrate would favour microbial growth compared with straw alone by providing necessary nutrients. It is suggested that adding concentrate would increase microbial colonization on straw and consequently improved dry matter and fibre degradation of poor quality substrate in the rumen. In our study, although rate of gas production was higher, the volume of gas production was lower by adding concentrate, and along with higher digestibility of dry matter, it is suggested that the fermentation efficiency would be improved by adding the concentrate. Doane et al. [48] also noted that gas production of the in vitro fermentation was negatively related to fibre degradation. The lower fibre content of the substrate and the increased fibre degradation by adding concentrate may explain the lower volume of gas production in our study. The positive response of in vitro digestion of poor-quality feed substrates to high fermentable carbohydrate addition suggests necessary consideration when needing to determine the potential digestibility of poor-quality roughage.

4. Conclusions

Several factors including inoculum source, gas venting system, substrate particle size and delivery, ratios of inoculum to buffer, and concentrate addition to media can influence the outcomes of fermentation in batch culture. The rumen inoculum plays a major role in the fermentation in batch culture. The purpose of the inoculum is to provide a suitable microflora to degrade a feed over time and to use the outcome to provide an estimate of rate or extent of feed digestion. The microbial activity of the inoculum can be considerably varied with animal

species (e.g., cattle versus sheep), diets, sampling schedule following feeding time, but the most important consideration is to ensure sufficient microbial activity in the inoculum and to reduce the variation of microbial activity among inocula. A means of reducing the variation, perhaps by increasing the number of donor animals and standardizing the inoculum collection time, is likely required. Many researches have been conducted to compare rumen fluid and faeces and aimed to develop an alternative to rumen fluid. The advantage of using faecal inoculum is primarily to reduce the requirement to rumen cannulated animals. However, it should be recognized that faecal and rumen inocula are slightly different. It appears that faeces have the potential to replace rumen fluid if long term in vitro end-point measurements are considered, whereas rumen fluid should be used if short-term or kinetic data are needed. Gas production that is main measurement in batch culture is highly adaptable and powerful research tools at present ruminant nutrition research. The discussion of different venting systems and substrate delivery methods is inconclusive. It suggests that other factors such as bottle size, headspace and type of feeds incubated could be interacted with these systems. The particle size of substrate incubated has consistent influence on rate and extent of feed digestion. The recommendation on the particle size of feed may be not easily provided and may depend on type of feed (e.g., concentrate versus roughage) and the objective of the study. Varying ratios of rumen fluid to buffer volume changes microbial activity in fermentation media, thus potentially alter rate of fermentation and lag time. The recommendation is to ensure sufficient microbial activity in the mixture of fermentation media without too much rumen fluid which may increase proportion of gas from inoculum over substrate. Finally, adding highly fermentable carbohydrate is helpful to maximize the fermentation of poor-quality feeds.

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