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# Evaluation of primary rumen epithelial cell incubation techniques in sheep

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**Abstract**

Objectives of this study were to determine if the number of cells incubated in primary rumen epithelial cell cultures affects production rates of metabolites and to establish an ideal mode of data expression in order to standardize the reporting criteria for primary cell incubation. Epithelial tissue was excised from five Suffolk × Dorset cross-bred sheep and subjected to serial tryptic digestion to isolate cells. Isolated cells were incubated for 90 min in 25 mM propionate and 10 mM butyrate at concentrations of 0.5, 1, 5, 10, 20 and 40 million cells per flask (total volume = 3 ml). Production of acetoacetate (ACAC), β-hydroxybutyrate (BHBA), lactate (LAC) and pyruvate (PYR) were measured. Data were expressed as either cell number, cell dry matter (DM) or cell crude protein (CP) alone or per epithelial wet tissue weight, body weight (BW) or metabolic BW to generate 12 different forms of data expression. Coefficients of variation (CVs) were calculated for all 12 modes of expression. Expressing data per cell number resulted in the lowest variation ($P < 0.01$) and data adjusted for metabolic BW had less variation than BW. ACAC concentrations were largest at 0.5 million cells per flask ($P < 0.05$) and there were no differences between 1, 5, 10 and 20, and only 40 differed from 0.5 and 5 million cells per flask. Concentrations of BHBA were largest at 1 and 5 million cells per flask, but were different ($P < 0.05$) only from 20 and 40 million cells per flask. LAC and PYR concentrations were largest at 1 million cells per flask, but no significant differences were found. Ratios of BHBA:ACAC were below one for the 0.5 million cells per flask indicating low mitochondrial redox potentials ($P < 0.05$). A suggested range of rumen epithelial cells to include in incubations is 5–20 million cells per flask. This range will minimize the potential for altered metabolite production caused by incubating large cell quantities as well as the experimental error associated with using low cell numbers. When rumen tissue is taken from animals of the same species, size and stage of development, data adjusted by cell number is preferred. However, it is recommended that metabolic BW, cell CP and cell DM be included to facilitate future comparison between laboratories and species.

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## 1. Introduction

It is has long been known that the rumen of a foregut fermenting animal is an important site for absorption of volatile fatty acids (VFAs), which are a principal source of energy to the animal. The in vitro study of rumen epithelial tissue metabolism

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has spanned several decades starting with isolation of whole ruminal papillae (Pennington, 1952) to the more recent isolation of the metabolically active cell layers (stratum basale and stratum spinosum) (Gálfi et al., 1981; Baldwin and Jesse, 1991).

Rumen epithelial morphology can be affected by the diet. Different diet types producing differing proportions of ruminal VFAs that affect the size and number of papillae. Butyric and propionic acids stimulate papillary growth and acetic acid causes papillary regression (Hofmann, 1988). The rumen epithelium is a stratified squamous tissue. Its outer most cell layer, the stratum corneum, lies in direct contact with the luminal contents of the rumen. This corneal layer is primarily composed of dead cells and is thought to act as a barrier separating harsh conditions and opportunistic organisms of the rumen from the rest of the body (Fell and Weekes, 1975). Diet also influences the degree of keratinization, thereby creating a thicker or thinner barrier, which can affect the rate of VFA transport (Gálfi et al., 1983; Nocek et al., 1984; Baldwin, 1998). The degree of keratinization depends on the physical aspects of the diet as well as the rate of cell death and turnover (Fell and Weekes, 1975; Nocek et al., 1980).

These concepts make the technique of isolation of ruminal epithelial cells developed by Baldwin and Jesse (1991) ideal because it leaves only the metabolically active cell layers which reduces the resultant complications due to differing rumen morphology that arise when comparing animals in various experimental situations. This, in turn, makes it possible to study metabolite interactions resulting from various dietary treatments, compare metabolism based on the anatomical location within the rumen, compare tissue metabolism between animals at different developmental stages and make metabolic comparisons across species (Baldwin and Jesse, 1992; Lane et al., 2000; Waldron et al., 2002). All of these involve the comparison of tissues with significantly different morphology. Waldron et al. (2002) utilized this technique to determine if the ruminal epithelial metabolism differed between sheep and steers. Rates of production for the measured metabolites were, however, adjusted by a per million cell basis to balance for differences in number of cells incubated for sheep and steers. This comparison generated uncertainty regarding the comparison of data generated from incubations containing different quantities of cells. To address this, Klotz et al. (2001) utilized cattle and the present study utilized sheep. When making comparisons between different animals or species, the expression of metabolite production based on cell number assumes cells are of the same size, degree of maturation, same protein content and that cell incubation concentration does not affect cellular metabolite production. The objectives of this study were to determine if the number of cells incubated in primary rumen epithelial cell cultures affects production rates of metabolites and to establish an ideal mode of expression in order to standardize the reporting criteria for primary cell incubations, thus making it easier to compare data among laboratories and species.

## 2. Materials and methods

### 2.1. Materials

Bovine serum albumin (BSA) # A-6003 and sodium salts of butyrate and propionate used for incubation substrates were from Sigma. Trypsin (1:250) and *N*-2-hydroxyethylpiperazine-*N*′-2-ethane sulfonic acid (HEPES) were from Gibco BRL (Life Technologies Inc., Grand Island, NY). All other chemicals were reagent grade compounds from Fisher Scientific (Fair Lawn, NJ), Mallinckrodt Chemical (Paris, KY) or Sigma.

### 2.2. Animals and diets

Animals used in this study were 1-year-old Suffolk × Dorset cross-bred sheep with an average weight of 74 kg (ranged between 50 and 91 kg). All sheep were fed the same diet and ration: CO-OP lamb grower/finisher hi-energy-bov (Tennessee Farmers Cooperative, Lavergne, TN) prior to slaughter.

### 2.3. Tissue collection

Sheep were slaughtered according to USDA specifications, stunning with a captive bolt followed by exsanguination. The abdomen was opened and the rumen was immediately excised. Tissue was collected and prepared as described by Baldwin and Jesse (1991).

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