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Further assessment of the protozoal contribution to the nutrition of the ruminant animal

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ABSTRACT

The flow of protozoa from the reticulo-rumen is lower than expected, due to ability of protozoa to prevent washout through sequestration on feed particles and the rumen epithelium. In order to estimate the distribution of protozoa within the reticulo-rumen and passage to the omasum, Czerkawski (1987) developed a model containing pools for the rumen liquid phase, rumen solid phase, and the omasum. This model was used to estimate loss of protozoa in the omasum as well as the amount of protozoal protein available to the animal in the lower gut. A number of assumptions were incorporated into the model, some of which appear unsupported by current research. This paper represents an update, revision, and re-evaluation of Czerkawski's model, where the assumptions that all protozoa in the 'attached' phase are in solid digesta, and that protozoa only leave the rumen in the liquid, have been relaxed. Therefore, the revised model allows for sequestration of protozoa on the rumen epithelium and protozoal passage with particulate outflow. Using experimental observations with inputs within biological limits, the revised model and Czerkawski's original model were verified. The effect of diet on the model was then assessed using inputs from a 100% forage diet and a 35–65% concentrate diet. The extent of sequestration was also varied from complete sequestration, to partial sequestration, and no sequestration. A sensitivity analysis was conducted through a linear regression of perturbed mean inputs versus outputs. The results from the revised model indicate that within the reticulo-rumen, the concentrate diet has a greater fractional flow rate of protozoa from the liquid to solid phase, but a lesser fractional flow rate back to the liquid phase, compared to the forage diet. As well, the concentrate diet has a slower net growth rate of protozoa in the attached phase, compared to the forage diet. In the omasum, the forage diet has a less negative net growth rate, compared to the concentrate diet. The forage diet was also associated with smaller loss of protozoa from the omasum. There are limited data from the omasum to be incorporated into the revised model, especially for quantity of protozoa in the omasum. Further research on quantification of protozoa in the omasum could strengthen the predictions made by the model. Despite this, the revised model found a loss of protozoa in the omasum similar to that suggested by Czerkawski's original model 65–73% versus 66%. The revised model results indicate that efforts to increase protozoal flow to the duodenum should focus on reduced sequestration and increased outflow rate from the rumen, although more research is needed to quantify protozoa in the omasum, and to investigate the role of sequestration onto the wall of the reticulo-rumen.

1. Introduction

The protozoa of the reticulo-rumen constitute a significant proportion of microbial biomass of the rumen, and are believed to play an integral role in digestion of feedstuffs, in addition to serving as a protein source for the animal. Moreover, rumen protozoa are rich in unsaturated fatty acids and account for between 30% and 43% of conjugated linoleic acid and 40% of the vaccenic acid reaching the duodenum (Yáñez-Ruiz et al., 2006). Protozoa can have a stabilizing effect on the rumen during high-starch feeding when rapid bacterial growth can occur (van Zwieten et al., 2008), but protozoa are capable of limiting microbial protein flow to the abomasum through bacterial predation and are associated with increased production of methane (Newbold et al., 2015).

The flow of protozoa to the abomasum has also been suggested to be lower than expected, considering the size of the rumen population (Jouany et al., 1988). Protozoa in the omasum have been shown to represent 6–64% of the protozoa in the rumen fluid (Collombier et al.,

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1984; Michalowski et al., 1986; Punia et al., 1992, 1984; Weller and Pilgrim, 1974), but a study by Sylvester et al. (2005) using real-time polymerase chain reaction (PCR), found the protozoal proportion of microbial biomass in the duodenum to be comparable to rumen. This brings into focus two limitations of previous research surrounding rumen protozoa. First, the use of molecular-based techniques, such as real-time PCR, has greatly improved our ability to quantify protozoa. However, more research comparing molecular and traditional methods is needed (Newbold et al., 2015). In addition, these techniques allow for comparison of protozoal quantities in different sections of the gastrointestinal tract. Therefore, it is feasible that a number of studies that used classical quantification techniques and markers (e.g. AEPA (Ling and Buttery, 1978; Whitelaw et al., 1984), and DAPA (Ling, 1990)) may have been providing highly variable results and under-estimating the size of the protozoal population due to decreased sensitivity of the analytical method. Second, the majority of protozoa studies focus on protozoa dwelling within the rumen fluid, disregarding any protozoa that may be sequestered on feed particles (Orpin et al., 1985) or the rumen epithelium (Abe and Iriki, 1989). It is the protozoa in the attached phase of the rumen environment that could contain the majority of protozoal cells. *In vitro* work by Czerkawski and Breckenridge, (1979a, 1979b) found the concentration of protozoa in the fluid phase to be only 10–20% of the protozoa in the attached phase. Also, a recent *in vivo* study found the concentration of protozoa in the fluid phase to be as low as 3.6% of the protozoa associated with feed particles on a forage diet (Hook et al., 2011a, 2011b). Rumen sequestration is not equal for all protozoal groups. Holotrich protozoa associate to feed particles after a meal mainly due to their strong chemotaxis toward sugars and then rapidly migrate to the ventral reticulo-rumen to avoid passage out of the rumen, whereas Entodiniomorphids also associate to feed due to chemotaxis toward glucose and peptides, but do not show the same affinity to the rumen wall (Diaz et al., 2014).

Czerkawski (1987) developed a model which contained three pools, representing protozoa in the liquid and solid phases of the rumen, and those in the omasum. The aim was to estimate the proportion of protozoa in the liquid and attached phases of the reticulo-rumen, as well as to approximate the flow of protozoa out of the reticulo-rumen. Using this model, loss of protozoa in the omasum is estimated, as is the amount of protozoal protein available to the animal in the lower gut. Czerkawski's intriguingly simple model is based on a number of key assumptions, including (i) the protozoal population of the rumen is confined to two pools, namely free suspension in the liquid and associated with solid digesta; (ii) kinetics is first-order and steady state conditions are assumed; (iii) there is no net growth of protozoa in free suspension; (iv) the flow of protozoa between the liquid phase and solid digesta is bi-directional such that the ratio of the forward and backward rate constants is inversely proportional to the ratio of the liquid and solid phase volumes; and (v) all protozoa exiting the rumen do so via the liquid phase. In this study we have updated, revised, and re-evaluated Czerkawski's model by relaxing two of the more equivocal aspects of these assumptions, namely all attached protozoa are associated with solid digesta and all protozoal passage from the rumen is via liquid outflow. The revised model allows for passage via particulate matter outflow and for sequestration against the rumen wall.

2. The revised model

The revised model is shown in Fig. 1 using Czerkawski's notation. It comprises three protozoal pools, two in the rumen and one in the omasum. The rumen pools are free-living protozoa associated with the liquid phase and attached protozoa, either attached to feed particles or sequestered against the rumen wall. There are currently insufficient data to facilitate the formation of a pool for each solid-associated and epimural protozoa, so these were combined into an attached phase. The flows between pools and out of the system are in the directions indicated. Kinetics are assumed to be first-order. Sequestration is accounted for implicitly by manipulating k_{20} (sequestration equals 100% of Q_2 when k_{20} equals zero, equals 0% when k_{20} equals the fractional passage rate of solid digesta).

The differential equations describing the pools are:

$$\frac{dQ_1}{dt} = \mu_1 Q_1 + k_{21} Q_2 - k_{10} Q_1 - k_{12} Q_1$$

$$\frac{dQ_2}{dt} = \mu_2 Q_2 + k_{12} Q_1 - k_{20} Q_2 - k_{21} Q_2$$

$$\frac{dQ_0}{dt} = \mu_0 Q_0 + k_{10} Q_1 + k_{20} Q_2 - k_0 Q_0$$

where t is time (d). In steady state these differential equations yield:

$$(\mu_1 - k_{10} - k_{12}) Q_1 + k_{21} Q_2 = 0 \quad (1)$$

$$(\mu_2 - k_{20} - k_{21}) Q_2 + k_{12} Q_1 = 0 \quad (2)$$

$$(\mu_0 - k_0) Q_0 + k_{10} Q_1 + k_{20} Q_2 = 0 \quad (3)$$

Eqs. (1)–(3) can be solved as follows. From assumption (iii) there is no net growth of protozoa in free suspension:

$$\mu_1 = 0 \quad (4)$$

Substituting Eq. (4) in (1) and re-arranging yields Eq. (5):

$$\frac{Q_1}{Q_2}\left(=\frac{C_1 V_1}{C_2 V_2}\right) = \frac{k_{21}}{k_{10} + k_{12}} \quad (5)$$

Adding Eqs. (1) and (2) then re-arranging gives Eq. (6):

$$\mu_2 = k_{10}\frac{Q_1}{Q_2} + k_{20} \quad (6)$$

Using Eq. (5) in (6) yields:

$$\mu_2 = \frac{k_{10} k_{21}}{k_{10} + k_{12}} + k_{20}$$

Using assumption (iv) gives Eq. (7):

$$\frac{k_{12}}{k_{21}} = \frac{V_2}{V_1} \quad (7)$$

Using Eq. (7) in (5) yields:

$$\frac{C_1}{C_2} = \frac{k_{12}}{k_{10} + k_{12}}$$

Re-arranging gives:

$$k_{12} = \frac{C_1}{C_2 - C_1} k_{10}$$

Re-arranging Eq. (7) yields:

$$k_{21} = \frac{V_1}{V_2} k_{12}$$

Re-arranging Eq. (3) gives:

$$\mu_0 = k_0 - k_{10}\frac{Q_1}{Q_0} - k_{20}\frac{Q_2}{Q_0}$$

i.e.

$$\mu_0 = \frac{k_0 C_0 V_0 - k_{10} C_1 V_1 - k_{20} C_2 V_2}{C_0 V_0}$$

In summary, if the concentrations C_0, C_1, C_2, volumes V_0, V_1, V_2, and passage rates k_0, k_{10}, k_{20} are known, the remaining fractional rates can be determined by applying the formulae:

$$k_{12} = \frac{C_1}{C_2 - C_1} k_{10}$$