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Manipulation of Proteasomal-Generated Peptides for Feed-Forward Activated (FFA) Boosting of Rumen Microbial Cell Protein (MCP) Synthesis: A Comment Paper

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Abstract: This paper discusses possible factors limiting microbial cell protein (MCP) synthesis in rumen microbial cells including their nutrient transport system, their proteasomal concentration for peptide feed-forward activation (FFA) and the energy supply from ATP/NADPH for proteomic cell functions. There is an oscillating pattern with diurnal feeding like a wave function in microbial numbers and their activities in producing end products from energy fermentation. This paper then discusses whether the peptide are at optimum concentrations with the feeding pattern. It mentions possible factors that impact peptide concentration on protein synthesis. These are the proteases in and from the feed material and microbially, the limits by transport systems into the cell's milieu and the limits for dietary preformed amino acids (PFAA) that are reached for rumen microbial cells. Rates of microbial cell protein (MCP) synthesis are limited by the half-life of mRNA transcripts, their functional attachment to their ribosomal units, the half-life depending on the transcriptional levels, rates of RNA exohydrolases and proteasomal concentrations and their rate of peptide generation acting via FFA as stipulated by the Protein Energy Theory for MCP synthesis. Manipulation of transcription factors (TF) is proposed here for proteasomal concentrations in the cell using earlier developed technology referred to as peptide nucleic acid (PNA) Vit B12-carriered biologics intracellularly. There is initial evidence showing that when an endoglucanase alone is genetically manipulated in a non-continuous culture system that with fermentation more ATP is produced with lactic acid end product. As proposed, it still has to be ascertained whether the manipulated ATP supply for energy would suffice to maintain cellular growth with the cellulases involved and whether the resulting proteasomal concentrations with their 'catalytic' peptides from proteins (damaged or in excess) in the cell would suffice to cause an effect on rumen MCP synthesis.

INTRODUCTION

The consideration of this paper surrounds the factors that impacts microbial cell protein (MCP) synthesis in the rumen and applying the concept of possible pleiotropy that can limit protein-based or proteomic cell functions. To take an e. g. would be the trade-offs the microorganism may face with genetically induced changes as with genetically modified organismal (GMO) and genetically regulatable organismal (GRO) classes of microbes that can result in changes as in: 1) nutrient acquisition via its membrane transport systems, and 2) as possibly discussed at length in this paper, achieving higher steady-state concentrations of proteasomes for peptide release possibly 'chaperoning' them, with feed-forward activation (FFA) and also for the cellulosomes of the microbial cell for the provision of supplying the requisite energy from the ATP and NADPH currencies of the cell. It is unknown how much more energy serves as a new steady-state to increase these proteomic cell functions. But there is evidence of changes in cellulase function as boosted by genetic recombination resulting in increased lactic acid fermentation from cellulose digestion to

sugars during the ensilage process (X. Wan et al., 2024), which is an indication to further conjecture that a new steady-state with plasmid recombinant genetics could be achieved in terms of end product from the increased steady-state levels of ATP.

CONSIDERING THE MILIEU FOR PEPTIDES FOR CELLULAR PROTEIN SYNTHESIS BY THE RUMEN MICROBIAL CELL

As a model system, the assumption is made here with diurnal feeding cycles for sheep with continuous conditions of rumen digestion proceeding with the rise and fall according to feeding. This represents a background, like a wave function, on which the cells multiply, and subside when feed substrate is short in supply in the rumen short of 'washing out'.

It has been recently demonstrated by Y. Hao et al. (2024) in their study that with different days of both high fibre and high concentrate that feed intake diurnally oscillates and that abundance by fast-reacting and slow-delayed spp. react likewise to feeding and so with their production of energy as indicated by total VFA and their relative cellulase activity as indicated by the acetate to propionate ratio.

The goal of researching this further is to establish that non-optimum conditions for certain major ruminal spp. hold which would be ideally examined carefully in an artificial rumen (e. g. Czerkawski's Rusitec ®) where sampling and its disruption to examine it microscopically can, together with a quantitative cell count, and to analyze the extracted and characterized peptide cellular milieu proteomically would over time of day with feeding with each spp. probed could demonstrate less than optimum peptide concentrations at the given feed intake level towards approaching peak amplitude and over the peak as it subsides to base of amplitude.

It is possible to postulate that the conditions of peptide supply in the interior cellular milieu and also given feed types, viz. high concentrate versus high forage, would be dependent on: 1) their protein content and availability when proteolyzed by proteases both from incoming feed plant substrate and those expressed by rumen microbial spp., 2) their influx through transmembrane transport systems as another limiting factor, and 3) their relevance and dependence on the 'saturation' point or limit of the cell's need or quota for dietary pre-formed amino acids (PFAA) versus the microbial cell's capacity to synthesize amino acids de novo.

TRANSCRIPTOMICS FOR THE STUDY OF TRANSCRIPT EXPRESSION LEVEL AND TRANSCRIPT STABILITY FOR ELEVATING PROTEIN SYNTHESIS

It is possible to measure the half-life of cellular transcripts of RNA in secs. to mins. and to quantify their active functional copies as associated with their ribosomes, which should be noted, as limiting the upper theoretical limit of cell protein synthesis or translation.

The idea is to identify the factors affecting transcript stability which are the rate of transcription or RNA expression levels and RNA degradation. And that in the life cycle of a transcript from attachment and functionality with a protein-making ribosome, as per unit protein molecule or subunit, followed by its end with digestion via hydrolases including any RNA exohydrolases as possible candidates to manipulate a transcript's half-life with specific reference to the known proteasomal subunits and their abundance and half-life in return

which will result as they function in more peptides from what are referred to as damaged or excess protein, a pool available for protein recycling in the cell, that can generate 'chaperoning' peptides, recently stated as part of the Protein Energy Theory (D. A. Flores, 2024) resulting in fast-forward activation (FFA) of enzymic translation by ribosomal protein polymerases.

In one study T. Esquerre et al. (2014) it was demonstrated that by controlling transcript stability that mRNA concentrations could be fine-tuned with changes in growth rate in microbes with E. coli.

Our interest in particular here is to manipulate via transcription factors (TF) for the mRNA transcripts of proteasomal subunits to change their concentration during growth in the cell cycle. The GRO-related technology is proposed using known PNA-B12 cellular biologic carriers tested thus far with E. coli spp. at the Center for New Technologies, Warsaw, Poland with the research group headed by J. Trylska et al. (2017).

THE ENERGY CURRENCY (ATP, NADPH) AS THE FIRST LIMITING FACTORS TO PROTEIN SYNTHESIS

In fibre-digesting microbial cultures, the first limiting factor for substrate would be lignocellulose, specifically cellulose itself, and the cognate enzymatic activities being the protein unit called the cellulosome which as an approach, attacks the substrate three ways via: 1) endoglucanases, 2) exoglucanases and 3) cellobiases (cf. cellobiose, a disaccharide unit) producing via the mitochondrial ATP and NADPH energy substrates for synthesis and catabolism via the pathways of the tricarboxyllic acid (TCA) cycle and electron transport pathway which can be manipulated with, as proposed here, all three enzymic activities although an endoglucanase alone was able to stimulate a non-continuous culture that degrades fibre and produces simpler units of sugars and ferments to the end product of lactic acid for purposes of ensilage fermentation (X Wan et al., 2024).

CONCLUSION

It still has to be concluded as to the outstanding issues of: 1) the need for the ATP pool supplied in the cellular milieu and the sufficiency in boosting of a cellulase alone or cellulase complement producing a reasonable rate of simple sugars available for fermentation, then producing ATP and NADPH to fuel the synthetic flux of proteasomal gene subunits, via transcription and then translated from their transcripts, and 2) whether this would result in boosted steady-state levels of peptides that are 'catalytic' in the stipulated fast-forward activational (FFA) mode for the upregulated synthesis of microbial cell protein (MCP) in microbes of the rumen stomach in livestock.

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