**Systematic review: Efficacy of exogenous enzymes for dairy calves**

**Protocol**

1. **Introduction**

The utility of exogenous enzymes (EE) to improve the sustainability and efficiency of ruminant production, through enhancing the hydrolytic capacity of the rumen and manipulating the rumen microbiome to produce favourable fermentation products and environmental outcomes, has been widely researched. Several reviews have been conducted on the use of EE in ruminants, finding varying, but generally positive, effects of EE on ruminant production, health, and environmental sustainability (Beauchemin et al., 2003; Meale et al., 2014; Sujani and Seresinhe, 2015; Arriola et al., 2017; Tirado-González et al., 2018). To this authors knowledge, there exists no systematic review of research examining the effect of EE in dairy calves. For the purposes of this review, dairy calves shall be considered as animals under 12 months of age, including the preruminant stage. EE may offer an avenue to enhance digestibility traits of young cattle, especially at the preruminant stage, where the physiological development of the rumen, and the microbial population that resides there in, is paramount for calf health and productivity (Davis and Drackley, 1998). A preliminary literature search has returned few reports of research examining the effects of EE in dairy calves, spanning at least half a century. Thus, systematically reviewing and compiling the existing literature should identify knowledge gaps and quantify the strength of evidence in favour of using EE in dairy calves.

1. **Objective**

The primary objective of this systematic review is to consolidate and evaluate the evidence to support the efficacy of exogenous enzymes to improve digestibility, physiological, and productivity traits of dairy calves. Results from all studies reviewed will be tabulated and discussed, with conclusions drawn from the data presented.

1. **Methods**
	1. **Databases and search terms**

This review will follow PRISMA guidelines (Page et al., 2021) for systematically reviewing literature. The following databases, search terms, and filters will be used to search for relevant literature:

* Web of Science (<https://login.webofknowledge.com>): exogenous AND enzyme AND (preruminant OR calf OR calves)
* ScienceDirect (<http://www.sciencedirect.com/>): Abstract/title/keywords search: (calf OR calves OR preruminant) AND (exogenous enzyme)
* PubMed (<https://pubmed.ncbi.nlm.nih.gov>): (calf OR calves OR preruminant) AND (exogenous enzyme)

Additional filters:

Text availability: “Abstract” + “Full text”

Species: “Other Animals”

Article language: “English”

* Google Scholar (<http://www.scholar.google.com/>): exogenous enzyme AND (calf OR calves OR preruminant). The top 100 hits from Google Scholar will be included.
	1. **Inclusion and exclusion criteria**

Literature will be included in the review according to the following selection criteria:

* Experimental animals must be dairy cattle breeds or dairy breed crosses. Studies using beef calves, other bovines, sheep, or goats will be excluded.
* Animals must be under the age of 12 months for the duration of the experimental period.
* *In vitro* digestibility studies will be included, providing the inoculum used is derived from animals pertaining to the previous selection criteria.
* Research articles must have an animal performance component. Studies using only blood, tissue, or organ assays will be excluded.
* Articles must be available in English.
* Email contact will be attempted with authors of closed access articles, if no response is received within a month, articles will be excluded.
* Studies that use EE in combination with other treatments such as probiotics or trace elements will be included but discussed separately to those using EE as standalone treatments.
* Any relevant reviews or short communications will be included.
	1. **Screening and secondary reviewing**

Initial title screening will be performed by the primary reviewer, with all literature produced from database searches inputted into an Excel workbook, and duplicate papers removed. The secondary reviewer will be randomly and blindly allocated 20 % of the reviewed papers, to ensure consensus on selection criteria. The same process will follow for main text screening and paper selection. Screening will take place in the format displayed in the figure below.

Identification

Screening

Eligibility

Inclusion

Records identified from database searches (**n = X**)

Records after duplicates removed (**n = X**)

Records screened by primary reviewer (**n = X**)

Records title screened by secondary reviewer (**n = X**)

Records excluded (**n = X**)

Records assessed by whole text by primary reviewer (**n = X**)

Records whole text screened by secondary reviewer (**n = X**)

Records excluded (**n = X**)

Records included in the review (**n = X**)

**Secondary reviewer instructions**

**Title and abstract screening**

Following the PRISMA guidelines, the searches displayed in the main protocol have now been performed, resulting in a total of 332 articles, once duplicates were removed. To ensure the primary reviewer is adhering to the inclusion/exclusion criteria, the secondary reviewer should screen 20 % of the articles and indicate whether they think they should be included (I) or excluded (E). Using a random number generator, 67 papers were selected and have been inputted into a separate excel spreadsheet where the secondary reviewer cannot view the primary reviewer’s decision to include or exclude. Initial screening will use the title and abstract of each article only. In the blinded Excel spreadsheet, the secondary reviewer should indicate I or E next to each paper. Once the secondary screening has been performed, both reviewers will compare results, and if there is disagreement for which articles should be included, discussion and refinement of selection criteria will take place.

**Whole text screening**

Following complete agreement between reviewers in papers to be included in initial screening, 27 articles have met the inclusion criteria to progress to whole text screening. Six of these articles have been randomly selected for whole text screening by the secondary reviewer. This screening must follow the same guidelines (PRISMA), and use the same inclusion/exclusion criteria, as set out in the main review protocol.

**References**

ARRIOLA, K. G., OLIVEIRA, A. S., MA, Z. X., LEAN, I. J., GIURCANU, M. C. & ADESOGAN, A. T. 2017. A meta-analysis on the effect of dietary application of exogenous fibrolytic enzymes on the performance of dairy cows. *Journal of Dairy Science,* 100**,** 4513-4527.

BEAUCHEMIN, K., COLOMBATTO, D., MORGAVI, D. & YANG, W. 2003. Use of exogenous fibrolytic enzymes to improve feed utilization by ruminants. *Journal of Animal Science,* 81**,** E37-E47.

DAVIS, C. L. & DRACKLEY, J. K. 1998. *The development, nutrition, and management of the young calf*, Iowa State University Press.

MEALE, S. J., BEAUCHEMIN, K. A., HRISTOV, A. N., CHAVES, A. & MCALLISTER, T. 2014. Board-invited review: opportunities and challenges in using exogenous enzymes to improve ruminant production. *Journal of animal science,* 92**,** 427-442.

PAGE, M. J., MCKENZIE, J. E., BOSSUYT, P. M., BOUTRON, I., HOFFMANN, T. C., MULROW, C. D., SHAMSEER, L., TETZLAFF, J. M., AKL, E. A. & BRENNAN, S. E. 2021. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *International journal of surgery,* 88**,** 105906.

SUJANI, S. & SERESINHE, R. 2015. Exogenous enzymes in ruminant nutrition: A review. *Asian Journal of Animal Sciences,* 9**,** 85-99.

TIRADO-GONZÁLEZ, D. N., MIRANDA-ROMERO, L. A., RUÍZ-FLORES, A., MEDINA-CUÉLLAR, S. E., RAMÍREZ-VALVERDE, R. & TIRADO-ESTRADA, G. 2018. Meta-analysis: effects of exogenous fibrolytic enzymes in ruminant diets. *Journal of Applied Animal Research,* 46**,** 771-783.