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Title:

Enhancing Protein Digestibility in Red Clover and Lucerne Seeds through Lactic Acid Fermentation

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Abstract: (Your abstract must use **Normal style** and must fit in this box. Your abstract should be no longer than 300 words. The box will 'expand' over 2 pages as you add text into it.)

Preparation of Your Abstract

1. The title should be as brief as possible but long enough to indicate clearly the nature of the study. Capitalise the first letter of the first word **ONLY** (place names excluded). No full stop at the end.

2. Abstracts should state briefly and clearly the purpose, methods, results and conclusions of the work.

Introduction: Clearly state the purpose of the abstract

Methods: Describe your selection of observations or experimental subjects clearly

Results: Present your results in a logical sequence

Discussion: Emphasize new and important aspects of the study and conclusions that are drawn from them

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Background: Red clover (*Trifolium pratense*) and lucerne (*Medicago sativa*) seeds are promising alternative protein sources due to their high crude protein content and rich nutrient profile. However, their potential is limited by the presence of anti-nutritional factors (ANFs) such as phytic acid, trypsin inhibitors, and chymotrypsin inhibitors. These compounds interfere with protein digestion, thereby reducing protein bioavailability. *Lactobacillus* fermentation has been proposed as a strategy to improve the nutritional quality of plant-based ingredients by enhancing protein hydrolysis, increasing the availability of bioactive compounds, and reducing ANFs.

Methods: Red clover and lucerne seed flours were fermented using three *Lactobacillus* strains, including *L. plantarum*, *L. acidophilus* and *L. casei*. ANFs, including phytic acid, trypsin inhibitory activity (TIA), and chymotrypsin inhibitory activity (CIA), were quantified using standard colorimetric assays. The INFOGEST protocol was used to evaluate *in vitro* protein hydrolysis (digestion), which was quantified by the o-phthaldialdehyde (OPA) method at three stages: before digestion, after the gastric phase, and after the intestinal phase.

Results: Fermentation significantly reduced ($p < 0.05$) all measured ANFs in both seed types. For example, TIA decreased by 33–82% depending on *Lactobacillus* strain and seed type. Before *in vitro* digestion, fermented samples exhibited an average four-fold increase ($p < 0.05$) in protein hydrolysis compared to unfermented controls. Furthermore, the gastric and intestinal protein hydrolysis values were significantly higher ($p < 0.05$) in the fermented samples, indicating improved digestibility compared to the unfermented controls.

Conclusion: *Lactobacillus* fermentation significantly enhanced the nutritional profile of lucerne and red clover seeds, reducing ANFs and improving protein bioavailability. The findings of this study suggest the potential application of *Lactobacillus* fermentation in developing nutritionally enhanced plant-based protein products using lucerne and red clover seeds.