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BRIEF COMMUNICATION: The Efficacy of Natural Substances to Protect Dietary Glutamine from Ruminal Degradation

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Abstract

In ruminants, the rumen protection of amino acids is required to prevent microbial degradation and increase the portion of undegradable dietary protein; therefore, increasing the total concentration of amino acids absorbed in the small intestine. The conditionally essential amino acid, glutamine, is vital to an array of biological processes throughout the body. The objective of the current study was to validate and optimise the use of two natural substances (NS1 and NS2) to rumen-protect glutamine from microbial degradation. Encapsulated glutamine has been tested in triplicate using an *in vitro* approach. It was concluded that, both natural substances provided protection to glutamine resulting in a 33.83% and 10.66% decrease in ammonia release with NS1 and NS2 respectively.

Keywords: amino acids, in vitro test, protein, rumen microorganisms, ruminants

Introduction

Balancing undegradable-dietary protein (UDP) and rumen-degradable protein (RDP) is crucial to maximise the efficiency of production in ruminants (Haddad et al. 2005; Sahoo & Walli 2008). Therefore, supplementing ruminants with rumen-protected amino acids (AAs) has received attention recently. Rumen protection is required to prevent microbial degradation allowing the supply of specific amino acids and increase the level of UDP. By increasing the UDP portion of the diet, these rumen-protected AAs can bypass the rumen and be absorbed within the small intestine and into circulation (Windmueller & Spaeth 1974; Hanson & Parsons 1997). Even though AAs are heavily involved in many biological functions, and their supplementation to livestock animals demonstrates several benefits, their polarity and hydrophilicity play an important role in defining the success and feasibility to rumen protect a diverse range of AAs.

Glutamine is an uncharged, polar AA because of the R side chain-containing an amine group. It has a pKa (COOH) of 2.17 and a pKa (NH_3^+) of 9.13. Glutamine is a conditionally essential AA and is involved in a multitude of biological processes (Häussinger & Sies 1984). These include immune function, specifically in the pro-inflammatory and some anti-inflammatory response mechanisms. Glutamine is also an important energy source for intestinal cells and aids in the growth and repair of enterocytes. This key AA is a major precursor for glutamate and is vital for foetal growth and survival during pregnancy in sheep (Vaughn et al. 1995). Glutamine has endless applications in optimising production in ruminants and is the chosen AA for this study.

The objective of this study was to determine the efficacy of two cost-effective natural substances (NSs) in protecting glutamine from microbial degradation in the rumen through *in vitro* analysis.

Materials and Methods

The rumen-protected glutamine substances, NS1 and NS2, were prepared under proprietary protocols. The protocols considered labour intensity and preparation time in manufacturing the rumen-protected glutamine to obtain a feasible and cost-effective process in a small-scale setting.

In Vitro Analysis

The rumen fluid was collected from an abattoir (TPL Meat Exports Pty Ltd) and transferred into a pre-warmed thermos. The *in vitro* methodology was adapted from Goering & Van Soest (1970) and Suh et al. (2022) with minor adjustments. The incubated bottles contained a negative control (blank), positive controls (glutamine only, NS1 only, and NS2 only), and the food-grade substance-encapsulated glutamine; all replicated in triplicate. The *in vitro* trial was conducted over a total of 480 minutes and sampling was conducted at 10 time points: 30, 60, 90, 120, 180, 240, 300, 360, 420 and 480 minutes in duplicate.

Ammonium Analysis

Nitrogen levels from the *in vitro* samples were indirectly measured using a colorimetric assay for ammonium determination. For this, 1 mL of each sample was added to microtubes containing 20 µL of 6 N HCl solution and then stored at –20°C prior to ammonium determination. Once defrosted, sample tubes are centrifuged at 1,107 g force or 5 minutes, and an ammonium standard solution (1,000 mg/L ammonium) was used to develop a standard curve. The ammoniacal nitrogen was determined by reacting the sample with sodium-salicylate and sodium nitroprusside. Then samples were exposed to a dichloroisocyanurate solution and the colorimetric detection was measured at 650nm by using the Multiskan plate reader (Bio-Rad Laboratories Inc., xMark™ Microplate Absorbance Spectrophotometer; Hercules, California, USA), 1 hour after all reagents were added (ammonium determination 11732; International Standards ISO 1997).

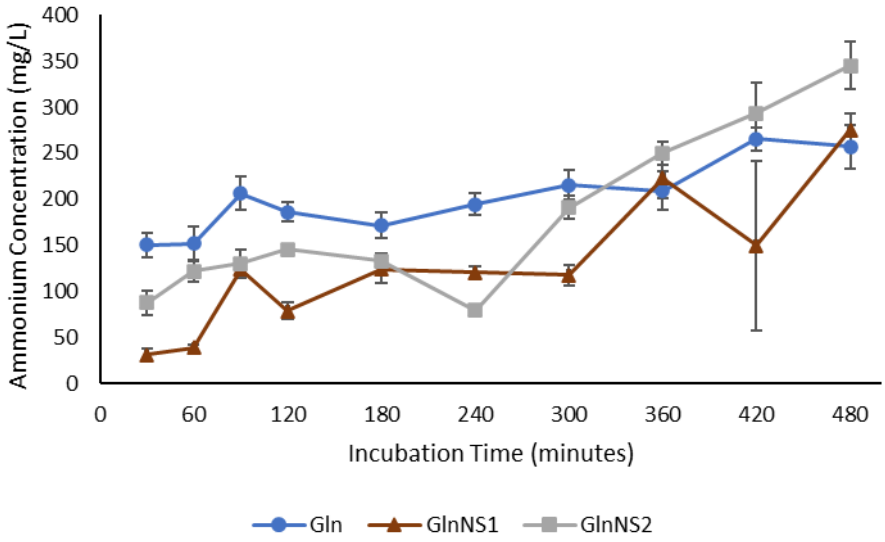
Statistical Analysis

Descriptive statistics and the area under the curve for each of glutamine-containing combination were calculated in SAS 9.4 using the PROC MEANS function.

Results

Glutamine (non-protected) had a stable release throughout the 480-minute incubation, while NS1 had prolonged efficacy in glutamine protection compared to NS2 (Figure 1). At 360 minutes of incubation, there was no difference between non-protected glutamine and glutamine protected with NS2.

Figure 1. Ammonium concentration (mg/L) of the incubated glutamine (Gln), encapsulated glutamine with natural substance 1 (GlnNS1), and encapsulated glutamine with natural substance 2 (GlnNS2) over 480 minutes *in vitro* incubation (mean ± SEM).



During 480 minutes of fermentation, the glutamine group (non-protected) released 9.24g of ammonia. During incubation, the bottle with glutamine protected with NS1 released 6.12g of ammonia that equates to 33.83% decrease in ammonia release. The bottle with glutamine protected with NS2 released 8.26g of ammonia that equates to 10.66% decrease in ammonia release.

Discussion

The study completed the objective to establish the efficacy of NS1 and NS2 to protect glutamine from microbial degradation in rumen fluid. NS1 resulted in a higher protective efficacy than NS2 as it had a 23.17% less ammonia released during the *in vitro* incubation. Both natural substances were economically feasible to manufacture, requiring minimal preparation and time-effective techniques.

There is dearth of information around the use of rumen-protected glutamine in ruminants and on methodologies and substances used to produce a rumen-protected glutamine supplement. A previous study investigated the use of formaldehyde 2% sprayed on glutamine and dried at room temperature to protect the AA from microbial degradation (Nemati et al. 2018). Depending on the volume of formaldehyde used, methodology is cost-effective being an important factor to consider for future commercial applications. Neither the efficacy nor the bioavailability of this supplement was revealed, therefore conclusions around its protection efficiency cannot be drawn.

Furthermore, current substances used to rumen-protect glutamine usually involve intellectual property and remain undisclosed. A rumen-protected glutamine product made by an Italian company, Ascor Chimici, was used in a study conducted by Caroprese et al. (2012) evaluating glutamine's effect on Friesian cow immune function. The glutamine supplement had a 40% bioavailability. This product is no longer commercially available, bringing into question the rumen protective efficacy and whether the cost of production was feasible. Additionally, rumen-protected glutamine produced by Wansheng Biological Co. Ltd. was used in a study conducted by Wang et al. (2022). This product has a high bioavailability of 65% and on average 25.3% rumen degradability (figures derived from their unpublished research).

The current study measured the efficacy of two natural substances in rumen-protecting glutamine therefore, to determine the bioavailability of the more efficient substance, NS1, an *in vivo* trial would be required. One third of the glutamine intake reaches blood circulation and the residual glutamine is metabolised by intestinal cells (Häussinger & Sies 1984). Therefore, blood glutamine analysis post feeding is a potential measurement for the evaluation of the amount absorbed through the small intestine and available to the body for use. Digesta passage rate and pH would also influence glutamine microbial degradation. Complete degradation of unprotected

glutamine in the rumen requires 36 hours (Suh et al. 2022). Different types of feed influence digesta passage rate; high concentrate diets are digested faster than high fibre diets. Differing diets can also influence pH which further impacts rumen microbiome (Vargas et al. 2023). In future studies where the previous diet of the ruminant is unknown, measuring rumen fluid pH would be beneficial.

In conclusion, natural substances 1 and 2 can be used in a cost-effective methodology to prevent partial microbial degradation of glutamine in the rumen. However, further investigation should be performed to evaluate if these natural substances are effective in increasing the levels of glutamine in the small intestine and the blood circulation of ruminants for metabolism.

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