In vitro evaluation of arginine bioavailability and ammonia generation in a new nutritional chew containing arginine

Luciana Rinaudi Marron
Oral Care, Early Research, Hard Tissue Health & BioSolutions, Piscataway, NJ

Background:
Arginine is an oral prebiotic. This amino acid is selectively metabolized by certain oral bacteria (arginolytic microorganisms such as *Streptococcus gordonii)* via the arginine deiminase pathway (ADS) to produce citrulline, ornithine, CO_2, adenosine triphosphate (ATP), and ammonia (Figure 1). Ammonia generation results in the increase of cytoplasmic and environmental pH, which favors the persistence of ADS-positive bacteria while being competitive against caries pathogens. pH modulation by alkali-generating substances such as arginine, aids in the neutralization of plaque acids and the prevention of tooth decay and therefore constitutes the natural defense mechanism against caries.

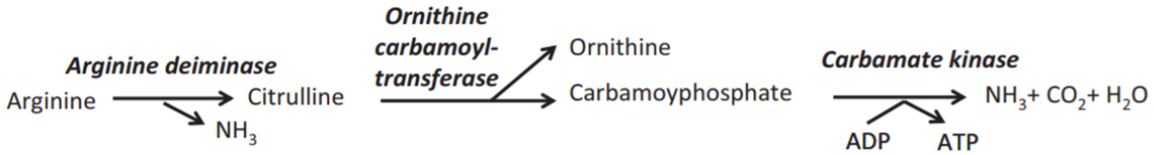

Figure 1. Graphical representation of the arginine deiminase pathway.

A new stackable nutritional chew containing arginine, xylitol and calcium has been developed to improve oral health. An *in vitro* planktonic ammonia assay was performed to confirm arginine's availability and ammonia production.

Experimental Procedure:
Test products

Treatment cells	Product ingredients
Control	LEMONGRASS 4x Calcium and Arginine Layer, 1x Vitamin B12 + Xylitol Layer, 1x Vitamin D3 Layer, 1 x No Inclusion Layer
No Xylitol	NO XYLITOL LEMONGRASS 4x Calcium and Arginine Layer, 1x Vitamin B12 Layer, 1x Vitamin D3 Layer, 1 x No Inclusion Layer
No Arginine	NO ARGININE LEMONGRASS 4x Calcium Layer, 1x Vitamin B12 + Xylitol Layer, 1x Vitamin D3 Layer, 1 x No Inclusion Layer

Chew slurries were prepared 1:1 in deionized water using a speedmixer for a final 0.1875% arginine concentration in the slurry.

Ammonia production
Briefly, an overnight culture of *S. gordonii* was thoroughly rinsed and resuspended in phosphate buffer (pH 4). These bacterial suspensions (normalized by cell weight) were incubated for 2 hours at 37°C in the presence of 0.1% sucrose and the treatments. Following incubation, the suspensions were centrifuged and the supernatants were used to quantify ammonia generation. A commercially available ammonia colorimetric assay (BioVision) was used according to the manufacturer's instructions. In this assay, ammonia reacts with phenol in the presence of hypochlorite to form indophenol, a highly colored product easily quantifiable by spectrophotometry at 670 nm.

Statistical analysis

Two independent tests were conducted with biological distinct samples, each with 3 technical replicates for a final n=6.

Two-way ANOVA with treatment as factor was conducted to determine whether significant differences existed between treatments. The treatment effect was considered significant if the p value was <0.05 (95% confidence level). If a significant difference was detected, a Tukey's multiple comparison test was used to assess pairwise differences among the treatments.

A two-sample t-test was also applied to compare the positive (Control) *vs.* negative (No arginine) treatments.

Results and Discussion:

Basal levels of ADS activity (ammonia production) were quantified in the negative control (no arginine chew) which contains no arginine (Table 1).

Treatment of *S. gordonii* suspensions with arginine-containing samples (control chew, and no xylitol chew) resulted in significantly higher ammonia generation compared to the negative control (Table 1). Ammonia production was not statistically different between the two arginine-containing products, suggesting the presence of xylitol at the level used in this nutritional chew is not detrimental to arginine utilization (Table 1).

Treatment	N	Ammonia (mM) (Mean ± StDev)	Grouping	
Control	6	0.11611 ± 0.00702	A	
No Xylitol	6	0.10803 ± 0.01137	A	
No Arginine	6	0.00134 ± 0.00596		B

Table 1. Quantification of ammonia production by *S. gordonii* bacterial suspensions. Grouping information using the Tukey method and 95% confidence. Treatments that do not share a letter are significantly different.

A two-sample t-test was conducted to compare ammonia generation upon treatment with the positive (Control) and negative (no arginine) treatments (Table 2).

There was a significant difference in the scores for control (M = 0.11611, SD = 0.00702) and no arginine (M = 0.00134, SD =0.00596) conditions; t(30.51) = 9, p = 0. These results indicate the control chew containing arginine led to significantly higher *in vitro* ammonia production than the chew without this oral prebiotic

Treatment	N	Mean	StDev	Difference	95% CI for Difference	T-Value	DF	P-Value
Control	6	0.11611	0.00702	0.11477	(0.10626, 0.12328)	30.51	9	0
No Arginine	6	0.00134	0.00596					

Table 2. Descriptive statistics

Conclusions:

Arginine degradation via the arginine deiminase pathway and the resulting production of ammonia is a natural defense mechanism against dental caries. Presence of ammonia upon treatment with this new arginine-containing nutritional chew confirms arginine's bioavailability and the active catabolism of arginine by an orally derived microorganism.

References:

Luciana Rinaudi Marr-00080