Synthesis, Characterization and Microencapsulation of the Antimicrobial Peptide Ctx(Ile^{21})-Ha for Necrotic Enteritis Control in Broiler Chickens

Rayssa Piton Rijo Costa[1]; Andrea B. Meneguin[2]; Mariana Q. Nascimento[3]; Cesar A. Roque-Borda[2]; Silvio R. Teixeira[4]; Marcus Chorilli[2] and Eduardo Festozo Vicente[1]

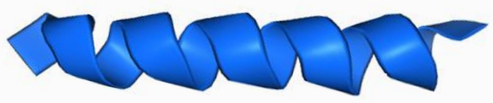
[1]*São Paulo State University (Unesp), School of Agricultural and Veterinary Sciences, Jaboticabal, 14884-900, São Paulo, Brazil.*
[2]*São Paulo State University (Unesp), School of Pharmaceutical Sciences, Araraquara, 14801-902, São Paulo, Brazil.*
[3]*Sapiens Artificial Intelligence in Microbiome Ltda., Jundiaí, 13212-321, São Paulo, Brazil.*
[4]*São Paulo State University (Unesp), School of Science and Technology, Presidente Prudente, 19060-900, São Paulo, Brazil.*
[5]*São Paulo State University (Unesp), School of Science and Engineering, Tupã, 17602-496, São Paulo, Brazil.*

eduardo.vicente@unesp.br
https://doi.org/10.17952/38EPS.2026.P234

INTRODUCTION

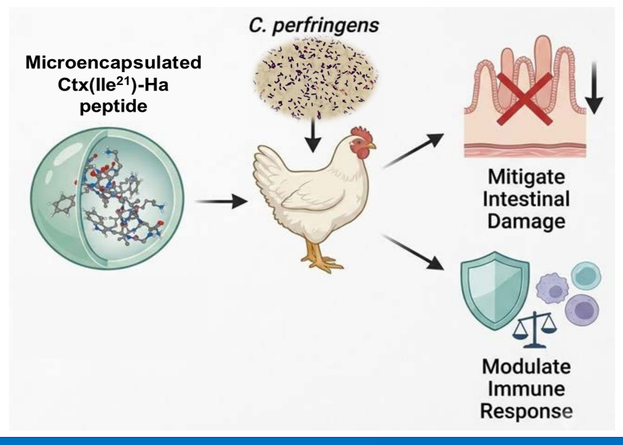
Necrotic enteritis, primarily caused by *Clostridium perfringens*, is an important challenge in poultry production and has become increasingly relevant following restrictions on the use of antibiotic growth promoters [1]. Antimicrobial peptides (AMPs) have emerged as promising alternatives due to their broad-spectrum antimicrobial activity and multiple mechanisms of action. Microencapsulation represents a promising strategy to protect AMPs during gastrointestinal transit and promote their controlled release at the intestinal site of action [2].





Antimicrobial Peptide Ctx(Ile^{21})-Ha
(*secondary structure predicted by AlphaFold3*)

OBJETIVES



Evaluate the potential of microencapsulated Ctx(Ile^{21})-Ha as a peptide-based strategy to mitigate intestinal damage and modulate immune responses in broiler chickens experimentally challenged with *Clostridium perfringens.*

METHODOLOGY

The antimicrobial peptide Ctx(Ile^{21})-Ha was synthesized by solid-phase peptide synthesis (SPPS), purified by reverse-phase high-performance liquid chromatography (RP-HPLC), and characterized by mass spectrometry. The peptide was microencapsulated using sodium alginate and subsequently coated with hydroxypropyl methylcellulose phthalate (HPMCP) to improve gastrointestinal stability and promote intestinal release. Encapsulation efficiency was determined by an indirect fluorescence method, while Fourier-transform infrared spectroscopy (FT-IR), X-ray diffraction (XRD), and thermal analysis were used to characterize the polymeric system and evaluate peptide incorporation and structural integrity. *In vitro* release assays were performed under simulated gastrointestinal conditions. Subsequently, an *in vivo* experiment was conducted in broiler chickens experimentally challenged with *C. perfringens*, in which the effects of dietary supplementation with microencapsulated Ctx(Ile^{21})-Ha antimicrobial peptide were evaluated through intestinal morphometry and serum protein profiling.

RESULTS

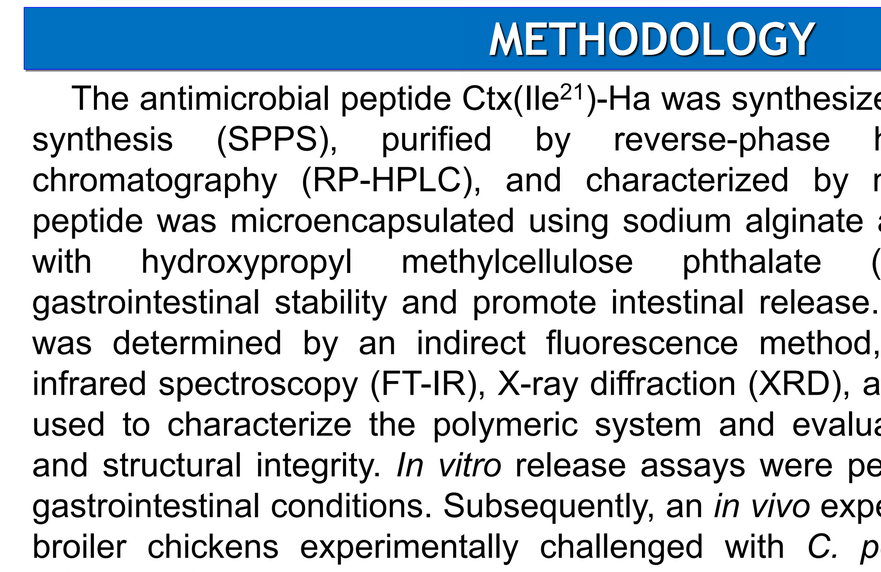


Figure 2. (A) Chromatogram profile of the synthesized Ctx(Ile^{21})-Ha antimicrobial peptide and **(B)** Mass spectrometry spectra. Retention time at approximately 18,5 min and purity greater than 95%. From the analysis of the LC-MS spectrum, it was able to detect the peaks of the [M+2H$^+$], [M+3H$^+$], [M+4H$^+$] and [M+5H$^+$] which confirm the material obtaining.

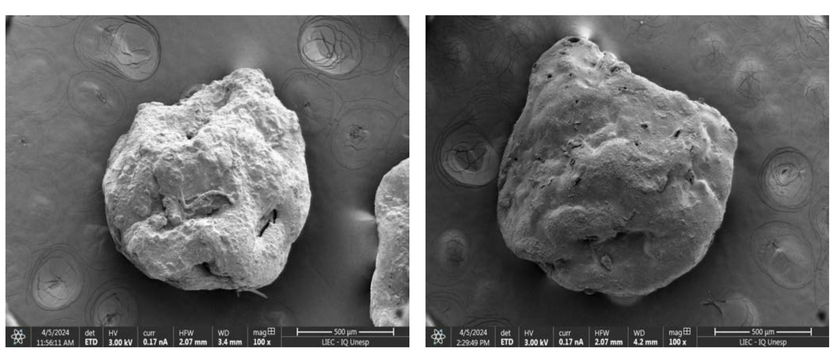


Figure 3. Scanning electron microscopy of coated microcapsules produced from microencapsulation solutions containing 100 mg (A) and 200 mg (B) of Ctx(Ile^{21})-Ha peptide.

RESULTS

Table 1. Release profile of coated microcapsules containing the antimicrobial peptide Ctx(Ile^{21})-Ha in simulated gastric solution (SGS) and simulated intestinal solution (SIS), according to the peptide loading.

Type of simulated solution	Mass	Measurement time (min–h)								
		0'	3'	5'	10'	15'	20'	30'	1h	2h
SGS (%)	100 mg	0%	0%	0%	0%	1%	0%	1%	2%	-
	200 mg	0%	0%	0%	1%	0%	0%	0%	1%	-
SIS (%)	100 mg	2%	9%	16%	25%	38%	52%	49%	77%	53%
	200 mg	1%	1%	23%	12%	8%	8%	12%	18%	20%

Table 2. Analysis of intestinal morphometry of the duodenum, jejunum, and ileum.

Intestinal segment	Treatments	Variables			*P*-value		
		Villus height (V)	Crypt depth (C)	Villus:crypt ratio (V:C)	V	C	V:C
Duodenum	T1	1.612^{a} ± 0.178	0.150^{c} ± 0.018	10.920^{a} ± 2.06	0.023	< 0.001	< 0.001
	T2	1.229^{b} ± 0.158	0.196^{bc} ± 0.030	6.345^{bc} ± 0.73			
	T3	1.310^{ab} ± 0.268	0.252^{a} ± 0.049	5.281^{c} ± 1.07			
	T4	1.204^{b} ± 0.276	0.219^{ab} ± 0.025	5.555^{c} ± 1.32			
	T5	1.385^{ab} ± 0.279	0.176^{bc} ± 0.049	8.121^{b} ± 1.63			
Jejunum	T1	0.764 ± 0.101	0.125^{b} ± 0.038	6.447^{a} ± 1.51	0.159	0.036	< 0.001
	T2	0.708 ± 0.101	0.188^{a} ± 0.054	3.934^{b} ± 0.79			
	T3	0.696 ± 0.139	0.176^{ab} ± 0.028	3.945^{b} ± 0.44			
	T4	0.627 ± 0.058	0.139^{ab} ± 0.030	4.628^{b} ± 0.81			
	T5	0.702 ± 0.061	0.147^{ab} ± 0.045	5.111^{ab} ± 1.44			
Ileum	T1	0.623 ± 0.120	0.096 ± 0.014	6.562^{a} ± 1.06	0.342	0.061	< 0.001
	T2	0.514 ± 0.075	0.133 ± 0.040	4.102^{b} ± 0.98			
	T3	0.548± 0.155	0.150 ± 0.050	3.835^{b} ± 0.93			
	T4	0.504 ± 0.102	0.138 ± 0.027	3.767^{b} ± 0.91			
	T5	0.562 ± 0.111	0.129 ± 0.024	4.417^{b} ± 0.90			

Data are presented as mean ± SD (standard deviation). Different letters among treatments within each intestinal segment indicate significant differences by Tukey's test at the 5% significance level (P < 0.05).

Table 3. Electrophoretic profile of total serum proteins* of broiler chickens subjected to the experimental treatments.

Variables	Treatments					SEM	*P*-value
	T1	T2	T3	T4	T5		
ProT (g/dL)	3.227	3.347	3.126	3.226	3.274	0.041	0.554
IgA (%)	0.067	0.085	0.076	0.087	0.079	0.002	0.106
CER (%)	0.049^{b}	0.080^{a}	0.0662^{ab}	0.069^{ab}	0.059^{ab}	0.003	0.008
TRANS (%)	0.114^{b}	0.174^{a}	0.170^{a}	0.189^{a}	0.170^{a}	0.007	0.004
ALB (%)	2.396	2.469	2.248	2.299	2.386	0.033	0.230
IgG (h.c.) (%)	0.067^{b}	0.084^{ab}	0.085^{ab}	0.111^{a}	0.085^{ab}	0.004	0.003
HAPTO (%)	0.012^{ab}	0.014^{ab}	0.017^{ab}	0.011^{b}	0.018^{a}	< 0.001	0.023
A1GA (%)	0.002	0.001	0.001	0.001	0.001	< 0.001	0.646
IgG (l.c.) (%)	0.020	0.023	0.022	0.029	0.041	< 0.001	0.152
PM (%)	0.377^{a}	0.245^{b}	0.256^{b}	0.273^{b}	0.287^{b}	0.010	< 0.001

** ProT – total proteins (g/dL); IgA – immunoglobulin A (%); CER – ceruloplasmin (%); TRANS – transferrin (%); ALB – albumin (%); IgG (heavy c.) – immunoglobulin G heavy chain (%); HAPTO – haptoglobin (%); A1GA – alpha-1-acid glycoprotein (%); IgG (light c.) – immunoglobulin G light chain (%); PM – molecular weight (%). Data are presented as mean, and SEM (standard error of the mean). Different letters among treatments for each protein indicate significant differences by Tukey's test at the 5% significance level (P < 0.05).*

- Ctx(Ile^{21})-Ha attenuated intestinal lesions resulting from the enteric challenge, optimizing the integrity and absorptive capacity of duodenal mucosa.
- Changes in serum markers (IgG, HAPTO, CER, TRANS) indicate a possible systemic immune control complementing the peptide antimicrobial activity.

CONCLUSIONS

Microencapsulated Ctx(Ile^{21})-Ha showed promising physicochemical stability, gastrointestinal protection, and controlled intestinal release, together with protective and immunomodulatory effects in broiler chickens challenged with *C. perfringens*. These findings support the potential of microencapsulated Ctx(Ile^{21})-Ha as a peptide-based alternative for the control of necrotic enteritis and as a promising strategy for reducing the reliance on antibiotic growth promoters in poultry production.

REFERENCES

[1] Brown K, Uwiera RRE, Kalmokoff ML, et al (2017) Antimicrobial growth promoter use in livestock : a requirement to understand their modes of action to develop effective alternatives. *Int J Antimicrob Agents* 49:12–24.

[2] Roque-Borda CA, Silva HRL, Crusca Junior E, et al (2021) Alginate-based microparticles coated with HPMCP/AS cellulose-derivatives enable the Ctx(Ile^{21})-Ha antimicrobial peptide application as a feed additive. *Int J Biol Macromol* 183:1236–1247.

ACKNOWLEDGEMENTS

FAPESP FUNDAÇÃO DE AMPARO À PESQUISA DO ESTADO DE SÃO PAULO
FAPESP Grant process numbers 2021/06706-9 and 2022/09379-1

CNPq Conselho Nacional de Desenvolvimento Científico e Tecnológico
Grant process number 311935/2022-2

unesp UNIVERSIDADE ESTADUAL PAULISTA "JÚLIO DE MESQUITA FILHO"