



## Characterization of a Coproduct from the Sea Cucumber *Cucumaria frondosa* and its Effects on Visceral Adipocyte Size in Male Wistar Rats

## Alan Ramalho <sup>1,2</sup>, Nadine Leblanc <sup>2</sup>, Marie-Gil Fortin<sup>3</sup>, André Marette <sup>2,4,5</sup>, André Tchernof <sup>1,4</sup> and Hélène Jacques <sup>1,2,\*</sup>

- <sup>1</sup> School of Nutrition, Faculty of Agricultural and Food Sciences, Université Laval, Québec, G1V 0A6, Canada; <u>alan.ramalho.1@ulaval.ca</u> (A.R.); <u>andre.tchernof@criucpq.ulaval.ca</u> (A.T.)
- <sup>2</sup> Institute of Nutrition and Functional Foods, Université Laval, Québec, G1V 0A6, Canada; <u>Nadine.Leblanc@fsaa.ulaval.ca</u> (N.L.); <u>Andre.Marette@criucpg.ulaval.ca</u> (A.M.)
- <sup>3</sup> Fisheries and Aquaculture Innovation Centre, Merinov, Gaspé, G4X 2V6, Canada; <u>Marie-Gil.Fortin@merinov.ca</u>
- <sup>4</sup> Quebec Heart and Lung Institute, Université Laval. Québec, G1V 4G5, Canada
- <sup>5</sup> Department of Medicine, Faculty of Medicine, Université Laval, Québec, G1V 0A6, Canada
- \* Correspondence: Helene.Jacques@fsaa.ulaval.ca

	Amino acid	Concentration	Amino Acid Score <sup>2</sup>
Essential	Histidine	$1.54 \pm 0.13$	1.03
	Isoleucine	$3.18 \pm 0.38$	1.06
	Leucine	$5.51 \pm 0.31$	0.93
	Lysine	$5.33 \pm 0.34$	1.18
	Methionine	$1.55\pm0.14$	0.97
	Phenylalanine	$3.31 \pm 0.29$	$1.58^{1}$
	Threonine	$4.11 \pm 0.21$	1.79
	Tryptophan	$0.57\pm0.05$	0.95
	Valine	$3.80 \pm 0.38$	0.97
Non-essential	Alanine	$4.71 \pm 0.25$	N.A.
	Arginine	$4.48\pm0.29$	N.A.
	Aspartic acid	$6.90\pm0.23$	N.A.
	Cysteine	$0.93 \pm 0.15$	1.55
	Glutamic acid	$10.4 \pm 0.5$	N.A.
	Glycine	$5.06 \pm 0.27$	N.A.
	Proline	$3.48 \pm 0.11$	N.A.
	Serine	$3.53 \pm 0.15$	N.A.
	Tyrosine	$2.69 \pm 0.17$	N.A.

**Supplementary Table S1**. Amino acid content of sea cucumber coproduct protein (mean  $\pm$  standard deviation; n= 4 replicates except for tryptophane where n=3) (g/100 g of protein).

<sup>1</sup> The amino acid score for phenylalanine is calculated using the sum of the contents of phenylalanine and tyrosine. <sup>2</sup> The amino acid scores were calculated according to the method outlined by the FAO/WHO/UNU using the adult indispensable amino acid requirements as determined by the FAO/WHO/UNU [92]. To calculate the scores, the amino acid content of the coproduct (mg amino acid /g protein) was divided by the requirement pattern of the same amino acid for adults (mg amino acid /g protein) [92]. N.A. not applicable



**Supplementary Figure S1**. Representative chromatogram of amino acids analysis by UPLC-PDA. In red, amino acids standards and in blue, a sample of amino acids after digestion of sea cucumber coproduct.



**Supplementary Figure S2**. Representative chromatogram of fatty acids analysis by GC-FID. In blue, a Supelco 37 Component FAME Mix and in red, a sample of fatty acids from sea cucumber coproduct.

**Supplementary Table S2**. Complete fatty acid profile of the lipids in the dried sea cucumber coproduct (mg/g lipid).

Fatty acid	Retention time (min)	Replicate 1	Replicate 2	Replicate 3
C6:0				
C8:0				
C10:0				
C11:0				
C12:0				
C13:0				
Unidentified 1	26.99	6.75	4.65	7.13
C14:0	29.67	4.91	5.46	4.44
Unidentified 2	29.97	9.5	10.83	9.73
Unidentified 3	32.74	56.21	69.03	58.31
C14:1n9 cis				
Unidentified 4	33.87	8.58	9.26	9.11
C15:0				
C15:1				
Unidentified 5	36.67	17.36	19.11	18.58
Unidentified 6	37.76	5.47	5.61	6.3
C16:0	39.27	8.43	8.38	9.36
C16:1n7 cis	42.07	68.57	73.71	72.69
C17:0				
C17:1				
C18:0	52.49	14.01	13.76	15.14
C18:1n9t				
C18:1n9c	55.52	12.88	13.17	13.5
C18:1n7 cis	56.13	14.21	13.97	15.27
C18:2n6 trans				
C18:2n6 cis	60.23			
C18:3n6				
C18:3n3	66.56			
C20:0	67.86	4.34	3.09	4.4
C20:1	71.05	2.38	2.05	2.73
C21:0				
C20:2n6	76.78	2.07	1.19	2.4
C20:3n6				
C20:4n6	83.12	5.01	4.24	4.9
C20:3n3	83.54			
C22:0	83.77			
C22:1n9	87.15	3.95	3.45	3.96
C20:5n3	89.56	145.37	148.30	155.57
C20:4				
C24:0	96.57			
C24:1	98.89	7.93	7.20	8.19
C22:5n3	101.56			
C22:6n3	103.33	7.48	7.13	7.43

Control

0.5%



**Supplementary Figure S3**. Representative histological samples of epididymal adipose tissue subregions fixed in buffered formalin and stained with hematoxylin and eosin from each diet group. The images have been reduced to 35% of their original size while preserving the aspect ratio. The images have not been altered in any other way.

Control











**Supplementary Figure S4**. Representative histological samples of epididymal adipose tissue subregions after being converted to 8-bit, having the background subtracted, the threshold adjusted, and converted binary. These subregions are the same as in Supplementary Figure S3.



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