frontiers

Effects of microalgae as dietary supplement on palatability, digestibility, fecal metabolites and microbiota in healthy dogs

Ana R. J. Cabrita[1*], Joana Guilherme-Fernandes[1], Maria Spínola[1†], Margarida R. G. Maia[1], Timur Yergaliyev[2,3], Amélia Camarinha-Silva[2,3], António J. M. Fonseca[1]

*** Correspondence:** arcabrita@icbas.up.pt

Table S1. Proximate composition (g kg^{-1} dry matter, DM), gross energy (MJ kg^{-1} DM), amino acids, macro- and trace elements, and fatty acids content (g kg^{-1} DM) of the microalgae species.

	Microalgae		
	Chlorella vulgaris	***Nannochloropsis oceanica***	***Tetradesmus obliquus***
Proximate composition			
DM, g/kg	978	988	982
Ash	98.9	340	110
Crude protein	439	246	411
Total lipids	97.9	140	83.8
Neutral detergent fiber	164	153	228
Acid detergent fiber	97.8	43.4	119
Starch	44.1	0.693	10.7
Gross energy	20.3	16.6	21.5
Essential amino acids			
Arginine	46.6	22.1	27.2
Histidine	9.51	5.19	4.61
Lysine	53.2	21.9	25.3
Threonine	28.0	14.5	21.7
Isoleucine	21.8	11.3	15.7
Leucine	39.7	20.7	28.4
Valine	29.1	14.8	20.2
Methionine	10.9	5.34	7.67
Methionine + cystine	12.8	6.24	9.16
Phenylalanine	29.9	14.3	21.3

Table S1 (cont.). Proximate composition (g kg^{-1} dry matter, DM), gross energy (MJ kg^{-1} DM), amino acids, macro- and trace elements, and fatty acids content (g kg^{-1} DM) of the microalgae species.

	Microalgae		
	Chlorella vulgaris	***Nannochloropsis oceanica***	***Tetradesmus obliquus***
Phenylalanine + tyrosine	57.4	27.2	38.9
Non-essential amino acids			
Cystine	1.94	0.90	1.49
Tyrosine	27.6	12.8	17.6
Aspartic acid + Asparagine	34.9	21.2	31.2
Glutamic acid + Glutamine	52.3	31.3	41.8
Alanine	35.6	17.7	28.6
Glycine	39.2	18.9	27.0
Proline	25.5	15.6	19.1
Serine	24.8	13.3	18.9
Macro elements			
Na	0.500	37.8	1.80
K	8.50	19.1	12.8
Mg	0.560	3.91	0.540
Ca	4.64	4.36	4.36
P	25.7	18.2	18.2
Ca:P ratio	0.181	0.239	0.239
Trace elements (mg kg^{-1} DM)			
Fe	644	300	2986
Mn	163	35.2	108
Cu	25.4	13.6	7.22
Zn	357	52.5	113
Se	0.170	1.36	0.310
Fatty acids			
C8:0	0.015	0.074	0.006
C10:0	0.073	0.123	0.089
C12:0	0.127	0.340	0.065
C14:0	1.21	4.79	1.51
C14:1 *n*-5	0.010	0.057	0.019

Table S1 (cont.). Proximate composition (g kg^{-1} dry matter, DM), gross energy (MJ kg^{-1} DM), amino acids, macro- and trace elements, and fatty acids content (g kg^{-1} DM) of the microalgae species.

	Microalgae		
	Chlorella vulgaris	***Nannochloropsis oceanica***	***Tetradesmus obliquus***
***iso*-C15:0**	0.671	0.388	0.254
***anteiso*-C15:0**	0.163	0.059	0.069
C15:0	0.114	0.281	0.221
***iso*-C16:0**	0.128	0.045	0.045
C16:0	11.8	18.8	11.7
C16:1 *n*-7	1.21	21.5	1.12
C16:1 *n*-9	2.03	1.02	2.53
C16:2 *n*-4	0.088	0.149	0.057
C16:3 *n*-4	0.022	0.174	0.030
C16:4 *n*-1	0.176	0.060	0.050
***iso*-C17:0**	2.80	0.716	2.57
C17:0	0.330	0.181	0.308
C17:1 *n*-8	0.096	0.115	1.47
C18:0	1.77	0.343	0.435
C18:1 *n*-7	1.36	0.518	1.45
C18:1 *n*-9	2.13	3.82	3.59
C18:2 *n*-6	10.4	2.77	3.69
C18:2 t*n*-6	0.083	0.038	0.157
C18:3 *n*-3	18.4	0.227	18.6
C18:3 *n*-6	0.045	0.263	0.256
C18:4 *n*-3	0.057	0.077	2.01
C20:0	0.016	0.031	0.040
C20:1 *n*-7	0.012	0.005	0.015
C20:1 *n*-9	0.035	0.043	0.068
C20:1 *n*-11	0.013	0.018	0.041
C20:2 *n*-6	0.055	0.058	0.016
C20:3 *n*-3	0.016	0.091	0.325
C20:4 *n*-3	0.020	0.045	BDL

Table S1 (cont.). Proximate composition (g kg^{-1} dry matter, DM), gross energy (MJ kg^{-1} DM), amino acids, macro- and trace elements, and fatty acids content (g kg^{-1} DM) of the microalgae species.

	Microalgae		
	Chlorella vulgaris	***Nannochloropsis oceanica***	***Tetradesmus obliquus***
C20:4 *n*-6	0.020	0.045	BDL
C20:5 n-3 (EPA)	0.106	17.8	0.169
C21:5 *n*-3	0.079	0.193	0.059
C22:0	0.096	0.078	0.227
C22:1 *n*-11	0.045	0.026	0.044
C22:2 *n*-6	0.010	0.017	0.014
C22:4 *n*-6	BDL	BDL	BDL
C22:5 *n*-3	BDL	0.023	BDL
C22:6 *n*-3 (DHA)	BDL	BDL	0.195
C24:0	0.199	0.050	0.235
C24:1 *n*-9	0.020	BDL	0.262
Sums and ratio			
SFA	16.7	25.9	16.5
MUFA	7.01	27.1	9.19
PUFA	32.8	27.5	28.9
PUFA *n*-3	18.7	18.5	21.3
PUFA *n*-6	10.8	8.01	4.24
***n*-6/*n*-3 ratio**	0.579	0.434	0.199

BDL: below the detection limit; SFA: sum of saturated fatty acids; MUFA: sum of monounsaturated fatty acids; PUFA: sum of polyunsaturated fatty acids; *n*-6/*n*-3 ratio: ratio of PUFA *n*-6 to PUFA *n*-3.

Table S2. Content of non-essential amino acids and fatty acids content (g kg^{-1} dry matter) of the reference and experimental diets with inclusion of microalgae in substitution of the reference diet.

	Diet										
	Reference		***Chlorella vulgaris***			***Nannochloropsis oceanica***			***Tetradesmus obliquus***		
			0.5%	**1.0%**	**1.5%**	**0.5%**	**1.0%**	**1.5%**	**0.5%**	**1.0%**	**1.5%**
Non-essential amino acids											
Cystine	4.56		4.55	4.53	4.52	4.54	4.52	4.51	4.54	4.53	4.51
Tyrosine	6.82		6.92	7.03	7.13	6.85	6.88	6.91	6.87	6.93	6.98
Aspartic acid + Asparagine	21.8		21.9	21.9	22.0	21.8	21.8	21.8	21.9	21.9	21.9
Glutamic acid + Glutamine	39.9		40.0	40.0	40.1	39.9	39.8	39.8	39.9	39. 9	39.9
Alanine	19.4		19.5	19.6	19.6	19.4	19.4	19.4	19.5	19.5	19.5
Glycine	26.7		26.8	26.8	26.9	26.7	26.6	26.6	26.7	26.7	26.7
Proline	24.8		24.8	24.8	24.8	24.8	24.7	24.7	24.8	24.7	24.7
Serine	15.5		15.5	15.6	15.6	15.5	15.5	15.5	15.5	15.5	15.6
Fatty acids											
C8:0	0.016		0.016	0.016	0.016	0.016	0.017	0.017	0.016	0.016	0.016
C10:0	0.082		0.082	0.082	0.082	0.082	0.083	0.083	0.082	0.082	0.082
C12:0	0.097		0.097	0.097	0.097	0.098	0.099	0.101	0.097	0.097	0.096
C14:0	0.910		0.912	0.913	0.915	0.930	0.949	0.969	0.913	0.916	0.919
C14:1 *n*-5	0.100		0.099	0.099	0.098	0.099	0.099	0.099	0.099	0.099	0.098
***iso*-C15:0**	0.023		0.026	0.029	0.032	0.024	0.026	0.028	0.024	0.025	0.026
***anteiso*-C15:0**	0.034		0.035	0.035	0.036	0.034	0.034	0.034	0.034	0.034	0.034

Table S2 (cont.). Content of non-essential amino acids and fatty acids content (g kg^{-1} dry matter) of the reference and experimental diets with inclusion of microalgae in substitution of the reference diet.

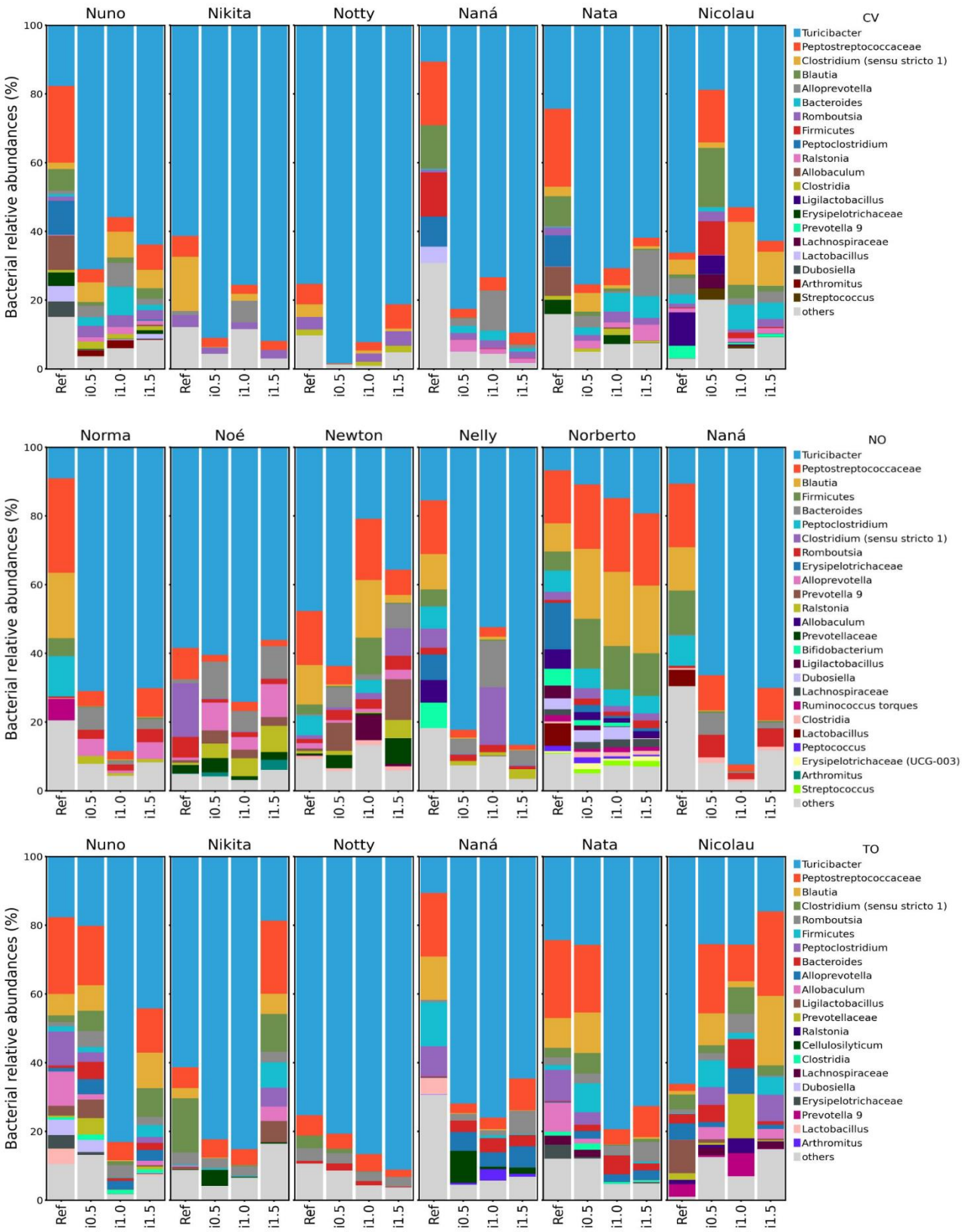
	Diet											
	Reference		***Chlorella vulgaris***			***Nannochloropsis oceanica***				***Tetradesmus obliquus***		
			0.5%	**1.0%**	**1.5%**	**0.5%**	**1.0%**	**1.5%**		**0.5%**	**1.0%**	**1.5%**
C15:0	0.127		0.127	0.127	0.127	0.128	0.128	0.129		0.127	0.128	0.128
***iso*-C16:0**	0.027		0.027	0.028	0.029	0.027	0.027	0.027		0.027	0.027	0.027
C16:1 *n*-9	0.288		0.297	0.305	0.314	0.292	0.295	0.299		0.299	0.310	0.322
C16:2 *n*-4	BDL		0.000	0.001	0.001	0.001	0.001	0.002		0.000	0.001	0.001
C16:3 *n*-4	BDL		0.000	0.000	0.000	0.001	0.002	0.003		0.000	0.000	0.000
C16:4 *n*-1	0.026		0.027	0.028	0.028	0.026	0.027	0.027		0.026	0.026	0.027
***iso*-C17:0**	0.091		0.091	0.092	0.092	0.091	0.091	0.090		0.093	0.094	0.096
C17:0	0.314		0.314	0.314	0.314	0.313	0.312	0.312		0.314	0.314	0.314
C17:1 *n*-8	BDL		0.000	0.001	0.001	0.001	0.001	0.002		0.007	0.015	0.022
C18:1 *n*-7	2.02		2.01	2.01	2.01	2.01	2.00	1.99		2.01	2.01	2.01
C18:2 t*n*-6	0.070		0.070	0.070	0.070	0.070	0.069	0.069		0.070	0.071	0.071
C18:3 *n*-6	0.070		0.070	0.070	0.069	0.071	0.072	0.073		0.071	0.072	0.073
C18:4 *n*-3	0.023		0.023	0.023	0.023	0.023	0.023	0.023		0.033	0.043	0.052
C20:0	0.210		0.209	0.208	0.207	0.209	0.208	0.208		0.209	0.209	0.208
C20:1 *n*-7	0.022		0.022	0.022	0.022	0.022	0.022	0.022		0.022	0.022	0.022
C20:1 *n*-9	0.449		0.447	0.445	0.443	0.447	0.445	0.443		0.447	0.445	0.443
C20:1 *n*-11	0.160		0.159	0.158	0.157	0.159	0.158	0.157		0.159	0.158	0.158
C20:2 n-6	0.222		0.221	0.220	0.220	0.221	0.220	0.220		0.221	0.220	0.219

Table S2 (cont.). Content of non-essential amino acids and fatty acids content (g kg^{-1} dry matter) of the reference and experimental diets with inclusion of microalgae in substitution of the reference diet.

	Diet										
	Reference		***Chlorella vulgaris***			***Nannochloropsis oceanica***			***Tetradesmus obliquus***		
			0.5%	**1.0%**	**1.5%**	**0.5%**	**1.0%**	**1.5%**	**0.5%**	**1.0%**	**1.5%**
C20:3 *n*-3	0.028		0.027	0.027	0.027	0.028	0.028	0.028	0.029	0.030	0.032
C20:4 *n*-3	0.024		0.024	0.024	0.024	0.024	0.024	0.024	0.023	0.023	0.023
C21:5 *n*-3	0.023		0.024	0.024	0.024	0.024	0.025	0.026	0.024	0.024	0.024
C22:0	0.114		0.114	0.114	0.114	0.114	0.114	0.114	0.115	0.115	0.116
C22:1 *n*-11	0.099		0.099	0.099	0.099	0.099	0.099	0.098	0.099	0.099	0.099
C22:2 *n*-6	0.006		0.006	0.006	0.006	0.006	0.006	0.007	0.006	0.006	0.006
C22:4 *n*-6	0.098		0.097	0.097	0.096	0.097	0.097	0.096	0.097	0.097	0.096
C22:5 *n*-3	0.054		0.054	0.054	0.053	0.054	0.054	0.054	0.054	0.054	0.053
C24:0	0.122		0.123	0.123	0.123	0.122	0.122	0.121	0.123	0.123	0.124
C24:1 *n*-9	0.064		0.064	0.063	0.063	0.063	0.063	0.063	0.065	0.066	0.067
Sums and ratio											
SFA	30.1		30.1	30.0	29.9	30.1	30.1	30.1	30.1	30.0	29.9
MUFA	35.0		34.8	34.7	34.6	34.9	34.9	34.9	34.9	34.7	34.6
PUFA	24.8		24.8	24.9	24.9	24.8	24.8	24.8	24.8	24.8	24.8
PUFA *n*-3	1.38		1.47	1.55	1.64	1.46	1.55	1.64	1.48	1.58	1.68
PUFA *n*-6	23.0		23.0	22.9	22.8	22.9	22.9	22.8	22.9	22.8	22.7
***n*-6/*n*-3 ratio**	16.7		16.6	16.5	16.5	16.6	16.5	16.5	16.6	16.5	16.4

BDL: below the detection limit; SFA: sum of saturated fatty acids; MUFA: sum of monounsaturated fatty acids; PUFA: sum of polyunsaturated fatty acids; *n*-6/*n*-3 ratio: ratio of PUFA *n*-6 to PUFA *n*-3.

Figure S1. Taxonomy barplots of reference diet and diets with the inclusion of *Chlorella vulgaris*, *Nannochloropsis oceanica*, and *Tetradesmus obliquus* at genus level or last available rank (if genus level was not assigned) by dogs.


Nuno
Nikita
Notty
Naná
Nata
Nicolau
Bacterial relative abundances (%)
Ref
i0.5
i1.0
i1.5
CV
Turicibacter
Peptostreptococcaceae
Clostridium (sensu stricto 1)
Blautia
Alloprevotella
Bacteroides
Romboutsia
Firmicutes
Peptoclostridium
Ralstonia
Allobaculum
Clostridia
Ligilactobacillus
Erysipelotrichaceae
Prevotella 9
Lachnospiraceae
Lactobacillus
Dubosiella
Arthromitus
Streptococcus
others
Norma
Noé
Newton
Nelly
Norberto
Naná
NO
Turicibacter
Peptostreptococcaceae
Blautia
Firmicutes
Bacteroides
Peptoclostridium
Clostridium (sensu stricto 1)
Romboutsia
Erysipelotrichaceae
Alloprevotella
Prevotella 9
Ralstonia
Allobaculum
Prevotellaceae
Bifidobacterium
Ligilactobacillus
Dubosiella
Lachnospiraceae
Ruminococcus torques
Clostridia
Lactobacillus
Peptococcus
Erysipelotrichaceae (UCG-003)
Arthromitus
Streptococcus
others
Nuno
Nikita
Notty
Naná
Nata
Nicolau
Bacterial relative abundances (%)
TO
Turicibacter
Peptostreptococcaceae
Blautia
Clostridium (sensu stricto 1)
Romboutsia
Firmicutes
Peptoclostridium
Bacteroides
Alloprevotella
Allobaculum
Ligilactobacillus
Prevotellaceae
Ralstonia
Cellulosilyticum
Clostridia
Lachnospiraceae
Dubosiella
Erysipelotrichaceae
Prevotella 9
Lactobacillus
Arthromitus
others