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Current uses and challenges of microalgae in animal feeding

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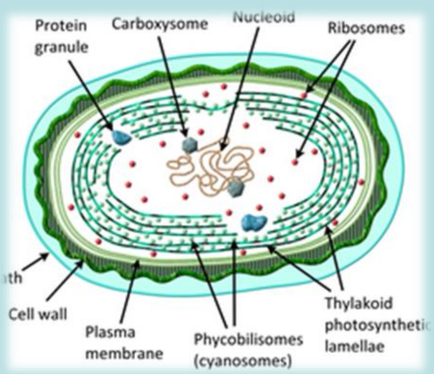
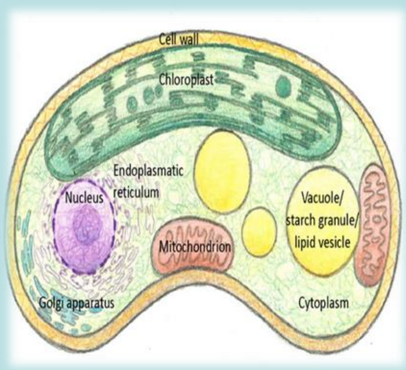
Microalgae definition

- Very diverse group of aquatic photosynthetic microorganisms (estimated as >100,000 species)
- Mostly eukaryotic organisms (diatoms, green algae and golden algae)
- But also some prokaryotic organisms (cyanobacteria or blue-green algae)



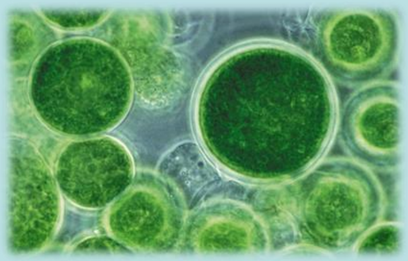
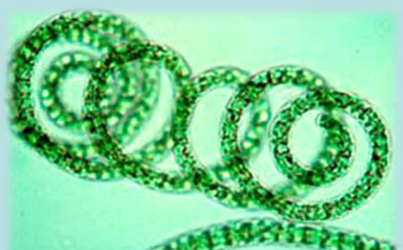
Prokaryotic microalga
(*e.g.* Spirulina)



Eukaryotic microalga
(*e.g. Chlorella*)

Some MA used for animal feeding

- Spirulina (*Arthrospira*) is rich in proteins (>60%)
- *Chlorella* is an important source of several nutrients
- *Isochrysis galbana* is rich in lipids (>20%, EPA+DHA)
- *Schizochytrium* is rich in lipids (>40%, DHA)
- *Nannochloropsis* is rich in lipids (>30%, EPA)


Potential benefits

- Richness in nutrients (proteins, lipids, carbohydrates, vitamins, minerals and antioxidants)
- Agriculture sustainability (alternative to staple food crops, mainly soybean and corn)
- Livestock production systems sustainability (novel sustainable raw materials, mainly for monogastrics)
- Environment sustainability (avoiding land degradation and saving water)
- Fish stocks sustainability (alternative to fatty fish as EPA+DHA source, mainly for aquaculture)

Nutritional composition of some species

Analysis	*Arthrospira platensis*	*Chlorella* sp.	*Isochrysis* sp.	*Schizochytrium* sp.
Crude protein (%)	60.3–65.8	37.7–47.8	27.0–45.4	12.1
Alanine (%)	5.4–6.5	4.6	2.4–3.2	0.6
Arginine (%)	4.0–4.9	3.1	2.1–2.5	1.2
Aspartic acid (%)	2.4–9.2	4.7	2.5–4.2	4.8
Cystine (%)	0.4–0.5	0.7	0.2–1.4	< 0.1
Glutamic acid (%)	5.7–10.7	5.8	3.0–4.6	1.2
Glycine (%)	1.8–5.2	3.4	1.8–2.6	0.4
Histidine (%)	1.5–2.7	2.2	0.6–0.9	0.1
Isoleucine (%)	4.2–4.4	2.0	1.5–1.8	0.2
Leucine (%)	5.5–8.0	4.7	2.7–3.9	0.8
Lysine (%)	2.9–3.0	4.0	1.8–2.5	0.4
Methionine (%)	1.2–1.6	1.2	0.8–1.4	< 0.1
Phenylalanine (%)	3.0–5.8	2.7	1.8–3.8	0.3
Proline (%)	2.0–4.0	2.5	1.8–2.4	0.3
Serine (%)	2.8–4.3	1.0	1.5–2.2	0.5
Threonine (%)	2.9–4.9	2.5	1.5–2.4	0.5
Tryptophan (%)	0.1–2.5	1.0	0.5–0.6	< 0.1
Tyrosine (%)	3.2–3.3	2.4	1.2–3.8	0.2
Valine (%)	4.2–4.6	3.0	1.8–2.4	0.3
Crude carbohydrates (%)	17.8–22.6	18.1–27.5	13.3–18.0	32.0
Crude fibre (%)	0.5–1.8	0.4–1.4	< 18.0	0.6
Crude fat (%)	1.8–7.3	13.3–20.9	17.2–27.3	38.0–71.1
DHA (g/kg)	< 3.0	< 26.0	< 34.0	104–204
EPA (g/kg)	< 2.5	< 4.0	< 3.5	< 20.0
Ash (%)	6.5–9.5	6.2–7.3	9.7–16.1	8.2

Madeira *et al.* 2017, Livest Sci 205 111

Effect on growth performance of ruminants

Microalga	Level in the diet (% dry matter) and experiment duration	Animal and initial weight/age	Main findings
Ruminants			
Arthrospira platensis	1.18% for 90 days	Dairy cows	Increase of body condition by 8.5-11%
Arthrospira platensis	10-20% for 6 weeks	Weaned lambs with 37.6 kg and 6 weeks	No effect on ADG Increase of body weight (10% dosage) Increase of backfat thickness (20% dosage)
Arthrospira platensis	0.01% for 5 weeks	Lambs with 46.5 kg	Increase of ADG, ADFI and final body weight Decrease of FCR
Isochrysis sp.	4% from 14.7 to 26.2 kg	Weaned male lambs with 14.7 kg	No effect on ADG, ADFI, FCR, carcass weight, carcass yield and backfat thickness
Schizochytrium sp	3.97% for 6 weeks	Dairy cows	Decrease of feed intake
Schizochytrium spp. (DHA-Gold extract)	1-3% for 18 weeks	Lambs with 22.7 kg	No effect on ADG, ADFI, FCR and carcass traits Increase of backfat thickness
Schizochytrium sp. (DHA-Gold extract)	1.92% for 6 weeks	Lambs with 34.8 kg and 3 months	No effect on ADG, FCR and carcass weight
Schizochytrium spp. (DHA-Gold extract)	3.89% from 16.3 to 26.7 kg	Lambs with 16.3 kg and 55.1 days	Decrease of ADG and feed intake Increase of slaughter age No effect on carcass traits
Schizochytrium sp. (DHA-Gold extract)	2% from 15.3 to 26.0 kg	Weaned male lambs with 15.3 kg and 7-8 weeks	Decrease ADG and feed intake Increase fattening period No effect on final live weight

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***Arthrospira* as feed additive in weaned lambs used as growth promoter**

Effect on meat quality of ruminants

Microalga	Level in the diet (% dry matter) and experiment duration	Animal and initial weight/age	Main findings
Ruminants			
Isochrysis sp.	4% from 14.7 to 26.2 kg	Weaned male lambs with 14.7 kg	No effect on chemical composition (fat, protein, moisture and ash), colour a Increase of MUFA, PUFA, CLA, EPA, DPA, DHA and TBARS at 7 days Decrease of vitamin E
Schizochytrium spp. (DHA Gold extract)	1-3% for 18 weeks	Lambs with 22.7 ± 3.90 kg	No effect on SFA and PUFA Increase of total *n-3* FA, EPA and DHA Decrease of *n-6/n-3* ratio
Schizochytrium sp. (DHA-Gold extract)	1.92% for 6 weeks	Lambs with 34.8 kg and 3 months	No effect on colour and pH Increase of cooking loss Increase of total *n-3* FA, EPA and DHA Decrease of *n-6/n-3* ratio
Schizochytrium spp. (DHA Gold extract)	3.89% from 16.3 to 26.7 kg	Lambs with 16.3 kg and 55.1 days	No effect on pH and a* Increase of L*, b* and TBARS Decrease of odour, flavour and overall acceptability
Schizochytrium sp.	1.8% for 20 weeks	Lambs with 35.3 kg and 9 months	No effect on colour, muscle fat, SFA and MUFA Increase of long chain *n-3* FA, total *n-3* FA, PUFA, EPA, DHA, PUFA/SFA rat TBARS Decrease of vitamin E and *n-6/n-3* ratio
Schizochytrium sp. (DHA-Gold extract)	2% from 15.3 to 26.0 kg	Weaned male lambs with 15.3 kg and 7-8 weeks	Increase of *n-3* FA, PUFA and DHA of neutral lipids and SFA, *n-3* FA and P polar lipids, and PUFA/SFA ratio Decrease of *n-6/n-3* ratio

Madeira *et al.* 2017, Livest Sci 205 111

Mainly used at low levels to increase meat/milk EPA+DHA

Effect on growth performance of pigs

Microalga	Level in the diet (% dry matter) and experiment duration	Animal and initial weight/age	Main findings
Pigs			
Arthrospira maxima	Biomass enriched with Cu from 20 to 105 kg	Piglets with 20.9 kg	No effect on ADG, ADFI and FCR
Arthrospira platensis	0.2% from 30.6 to 96.4 kg	Fattening pigs with 30.6 kg and 85 days of age	Increase of ADG and FCR No effect on backfat thickness
Arthrospira platensis and *Chlorella vulgaris*	1% for 14 days	Weaned piglets with 9.1 kg and 28 days	No effect on ADG, ADFI and FCR
Chlorella spp.	0.0002% from 30-55 kg	Female pigs with 30 kg and 3 months	No effect on ADG, body weight, hot carcass weight, lean muscle thickness and backfat thickness
Chlorella vulgaris	0.1-0.2% for 6 weeks	Growing pigs with 26.6 kg	Increase of ADG (0.1% dosage) No effect on ADFI, G:F and body weight
Schizochytrium sp.	1.10-5.51% from day 79 to 106 0.39-1.94% from day 107 to 120	Weaned castrated male swine with 9.07 kg	No effect on ADFI, body and organs weight Increase of ADG and FCR
Schizochytrium sp.	0.25% for 8 weeks 0.50% for 4 weeks	Barrows with 118 kg	No effect on ADG, FCR and backfat thickness

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Improved health status of weaned piglets

Contrasting impacts on growth as feed additive

Effect on meat quality of pigs

Microalga	Level in the diet (% dry matter) and experiment duration	Animal and initial weight/age	Main findings
Pigs			
Arthrospira platensis	0.2% from 30.6 to 96.4 kg	Fattening pigs with 30.6 kg and 85 days	No effect on ash, protein, colour, pH, cooking loss and tenderness Decrease of intramuscular fat
Chlorella spp.	0.0002% from 30-55 kg	Female pigs with 30 kg and 3 months	No effect on colour, pH, TBARS, cooking loss and drip loss
Schizochytrium sp. (DHA-Gold extract)	0.25% for 8 weeks 0.50% for 4 weeks 0.25% for 4 weeks	Barrows with 118 kg	No effect on chemical composition, colour and pH Increase of DHA Decrease of *n-6/n-3* ratio
Schizochytrium sp.	0.3-1.2% for 45 days	Female pigs with 75 kg	No effect on proximate composition (dry matter, fat and protein), colour, [illegible] TBARS Increase of EPA and DHA

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Mainly used as feed additive to increase meat EPA+DHA

Effect on growth performance of poultry

Microalga	Level in the diet (% dry matter) and experiment duration	Animal and initial weight/age	Main findings
Poultry			
Arthrospira sp.	4-8% for 16 days	Male chicks with 678 g and 21 days	No effect on ADG
Arthrospira platensis	1.5-2.5% for 4 weeks	Hens with 1.5 kg and 63 weeks	No effect on ADFI and FCR
Arthrospira platensis	6-21% for 21 days	Broiler chickens with 30 weeks	No effect on ADG, ADFI and FCR
Arthrospira platensis	0.5-1.5% for 36 days	Broiler chicks 1 day-old	Increase of ADG (1% dosage) Decrease of FCR (1% dosage)
Arthrospira platensis	0.5-1% for 42 days	Chicks with 49 g	No effect on ADG and FCR
Chlorella sp.	0.00003% for 42 days	Broiler chicks day-old	Increase of ADG No effect on F:G
Chlorella vulgaris	0.07-0.21% for 42 days	Broiler chicks day-old	Decrease of FCR No effect on ADG
Chlorella sp.	1% for 28 days	Male broiler chicks day-old	Increase of ADG No effect on ADFI and FCR
Chlorella sp.	1.25% for 25 to 39 weeks	Laying hens with 25 weeks	Decrease of FCR No effect on feed intake
Chlorella sp.	0.1-0.2% for 42 days	Male Pekin ducks day-old	Increase of ADG and feed intake No effect on G:F
Porphyridium sp.	5-10% for 10 days	Chickens with 30 weeks	No effect on body weight Decrease of ADFI
Schizochytrium JB5	0.1-0.2% for 35 days	Broiler chicks with 42.6 g	No effect on ADG, ADFI and FCR
Schizochytrium sp. (DHA-Gold extract)	3.7-7.4% from day 21 to 35	Broilers with 21 days	No effect on FCR and carcass yield Increase of ADG (7.4% dosage), ADFI (7.4% dosage)
Schizochytrium sp. (DHA-Gold extract)	7.4% from day 21 to 35	Broilers with 21 days	Increase of ADG, ADFI and carcass yield Decrease of FCR

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Contrasting impacts on growth for different inclusion levels

Effect on meat quality of poultry

Microalga	Level in the diet (% dry matter) and experiment duration	Animal and initial weight/age	Main findings
Poultry			
Arthrospira sp.	4-8% for 16 days	Male chicks with 678 g and 21 days	Decrease of L* (4% dosage) from *semitendinosus* and *sartorius* muscles Increase of a* (4% dosage) from *Psoas superficialis* and *Psoas sartorius* musc b* (8% dosage) from all muscles
Arthrospira platensis	0.5-1% for 42 days	Chicks with 49 g	No effect on SFA, MUFA, PUFA, EPA, DHA and TBARS in breast meat Increase of PUFA, EPA and DHA in thigh meat Decrease of MUFA in thigh meat
Chlorella sp.	0.00003% for 42 days	Broiler chicks day-old	Decrease of TBARS
Chlorella vulgaris	0.1-0.2% for 42 days	Male Pekin ducks day-old	Increase of b*, pH and shear force in breast meat and L* and b* in leg me
Schizochytrium sp.	2.8-5.5% for 3 weeks	Male broilers with 4 weeks	Increase of SFA (5.5% dosage), total *n-3* FA, EPA, DHA and TBARS Decrease of total *n-6* PUFA and flavour scores
Schizochytrium JB5	0.1-0.2% for 35 days	Broiler chicks with 42.6 g	Increase of oleic acid, USFA, total *n-3* FA and DHA Decrease of stearic acid (0.1% dosage), SFA, SFA/USFA ratio and *n-6/n-3*
Schizochytrium sp. (DHA Gold extract)	3.7-7.4% from day 21 to 35	Broilers with 21 days	No effect on colour, pH, cooking loss, shear force and MUFA Increase of SFA, total *n-3* FA, LCPUFA, EPA, DHA, *n-6/n-3* ratio and TBAR Decrease of total *n-6* FA, vitamin E, flavour and overall acceptability
Schizochytrium sp. (DHA Gold extract)	7.4% from day 21 to 35	Broilers with 21 days	No effects on pH and cooking loss Increase of SFA, total *n-3*, LCPUFA, EPA, DHA, off-flavour and TBARS Decrease of MUFA, total *n-6*, PUFA/SFA ratio, *n-6/n-3* ratio, vitamin E, flav overall acceptability

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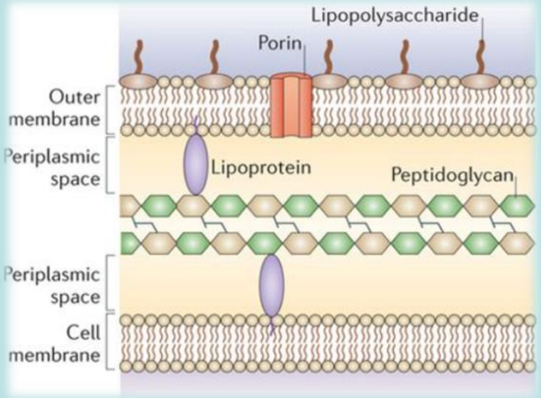
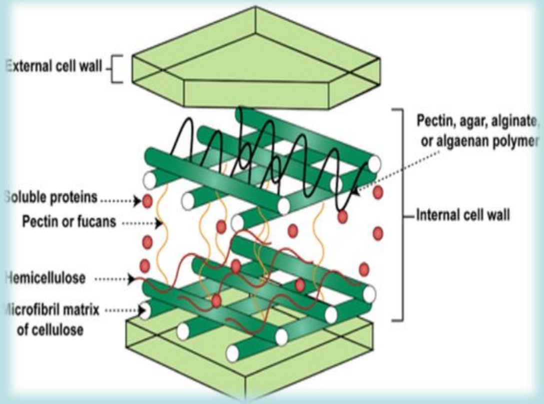
Mainly used at low levels to increase meat yellowness and EPA+DHA

Current use in animal feeding is limited

- MA have interesting properties for animal feeding
- Mainly used in fish farming (aquaculture)
- Feed additive to improve product quality (colour and n-3 LCPUFA)
- Feed additive to improve young animals health (prebiotic effect)
- Are largely indigestible (monogastrics) due to their complex and recalcitrant cell walls



Prokaryotic MA cell wall

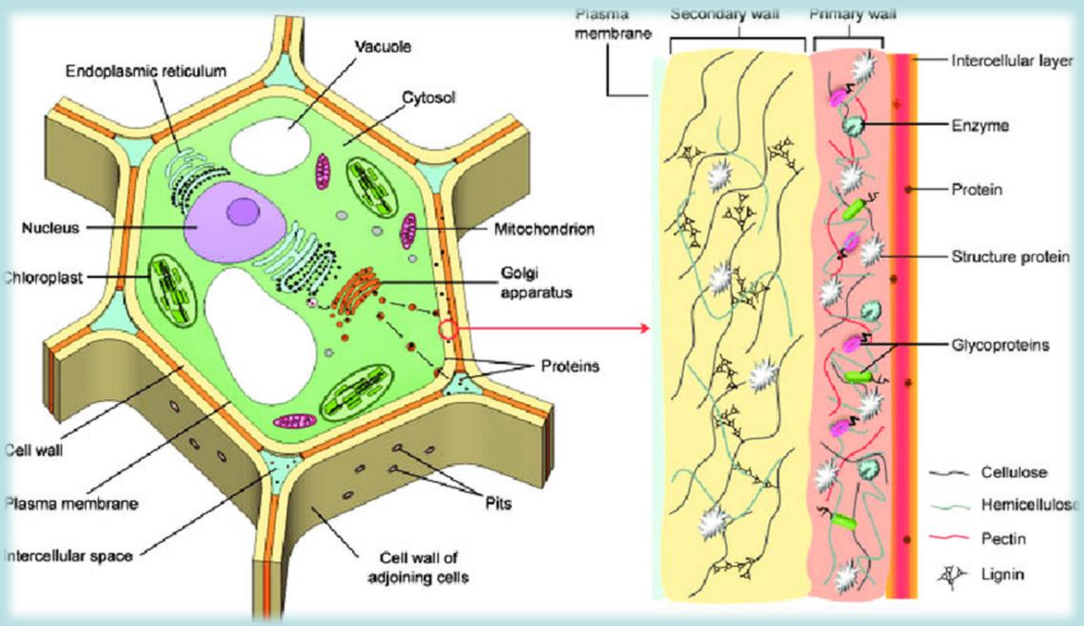


Eukaryotic MA cell wall

Challenge to improve digestibility

- These cell walls are distinct from those of terrestrial plants

- New enzymes are needed for MA cell wall degradation

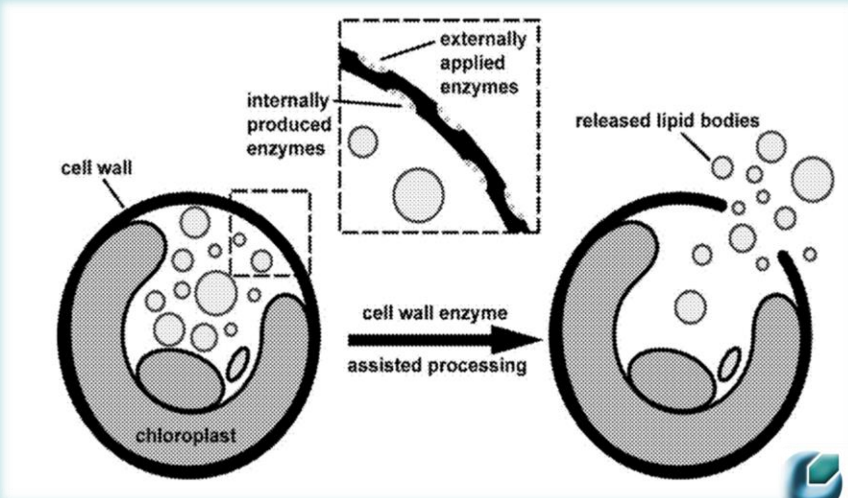


Typical plant cell wall

Commercial mixtures of carbohydrate-active enzymes for cereal-based diets (monogastrics):

- Rovabio® (Adisseo)
- Econase® (AB Vista)
- Naturgrain® (BASF)
- Ronozyme® (DSM)

Enzymatic valorisation of microalgae



CAZymes: www.cazy.org

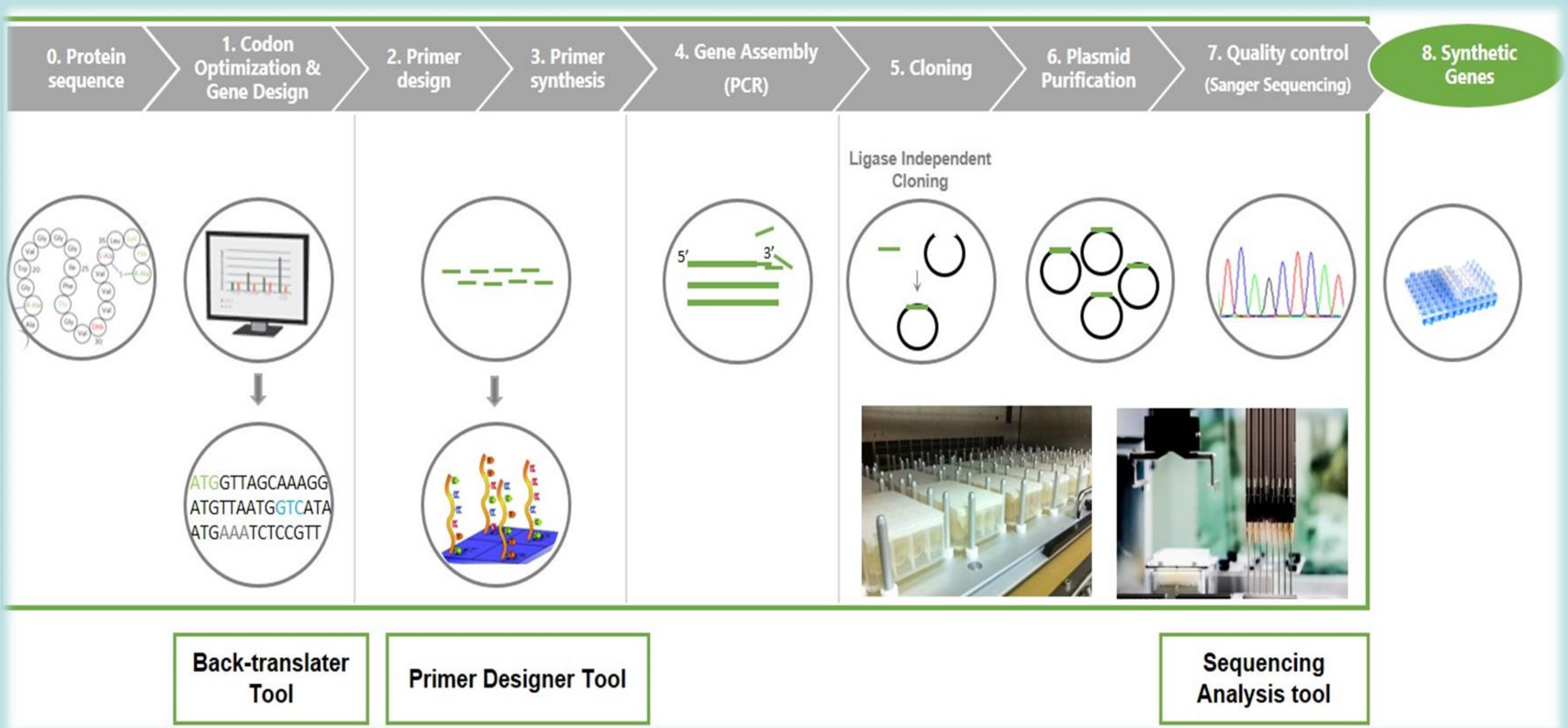
Development of an HTP approach to assess cell wall disruption by enzymatic digestion

Pre-selection of 200 CAZymes and sulfatases (marine origin)

ID	Name	Category	E.C	Main Substrate	Organism	Catalytic Domain	Mw (kDa)	Protein Sequence
4	Licheninase	1,3-1,4-β-Glucanases	3.2.1.73	1,3-1,4-β-glucans	*Clostridium thermocellum*	GH16	26,72	MKNRVISLLMASLLLVLSVIVAPFYKAEAATVVNTPFVAV
5	Cellulose 1,4-β-cellobiosidase	Cellobiohydrolases	3.2.1.91	Phosphoric acid-swollen cellulose, Avicel and others forms of insoluble cellulose	*Clostridium cellulolyticum*	GH48	80,21	MSKNFKRVGAVAVAAAMSLSIMATTSINAASSPANKVY
6	Glucan endo-1,3-β-D-glucosidase	1,3-β-Glucanases	3.2.1.39	1,3-β-glucans such as laminarin	*Clostridium thermocellum*	GH81	82,63	MLKKKTIISLVLAAIILGSIIQPFEIANAQYYREGTGSYTVVL
7	Chitinase	Chitinases & Chitosanases	3.2.1.14	Chitin and pNP-chitotriose	*Clostridium thermocellum*	GH18	43,93	MKKIPLLMLLSAIIFLSLHPTLSYAQDDSLPTKRIVGYFAE
8	Cellulose 1,4-β-cellobiosidase	Cellobiohydrolases	3.2.1.91	Phosphoric acid-swollen cellulose, Avicel and others forms of insoluble cellulose	*Clostridium thermocellum*	GH48	81,62	MVKSRKISILLAVAMLVSIMIPTTAFAGPTKAPTKDGTSY
9	-	Mini-Cellulosome	3.2.1.73	Variety of cellulosic substrates	*Clostridium thermocellum*	3×GH16 + Coh-Coh-Coh	Doc: 34.94	MKNRVISLLMASLLLVLSVIVAPFYKAEAATVVNTPFVAV
10	Endo-β-N-acetylglucosaminidase	Acetylglucosaminidases	3.2.1.96	Mammalian high mannose N-glycans (HMNG), such as Man9GlcNAc2	*Bacteroides thetaiotaomicron VPI-5482*	GH18	50,35	DDLEVGKNIDESAYSGIYENNAYLRDGKSNLVSKVVELHG
11	Cellulase	Cellulases	3.2.1.4	1,3-1,4-β-glucans and soluble 1,4-β-glucans	*B. subtilis LN*	GH16	26,12	MPYLKRVLLLLVTGLFMSLFAVTSTASAQTGGSFFDPFN
12	β-1,4-glucanase	Cellulases	3.2.1.4	Amorphous cellulose (PASC)	*Clostridium thermocellum F7*	GH48	74,8	MKLRYKVRRRRRIITCCGIIAAVIVVSTLIITIKNSFKPSRQS
13	Laccase	Laccases	1.3.3.5	2,20-azinobis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS)	*Bacillus subtilis subsp. subtilis str. 168*	AA NC	60,55	MTLEKFVDALPIPDTLKPVQQSKEKTYYEVTMEECTHQL
14	Unsaturated rhamnogalacturonyl hydrolase	Rhamnogalacturonases	3.2.1.172	Unsaturated rhamnogalacturonan (RG)	*B. subtilis subsp. subtilis str. 168*	GH105	43,49	MGSMDQSIAVKSPLTYAEALANTIMNTYTVEELPPANR
15	Laccase / Multicopper oxidase	Laccases	1.10.3.-	2,20-azinobis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS)	*Escherichia coli K-12 MG1655*	AA NC	55,47	MQRRDFLKYSVALGVASALPLWSRAVFAAERPTLPIPDL
16	Unsaturated rhamnogalacturonyl hydrolase	Rhamnogalacturonases	3.2.1.172	Rhamnogalacturonan oligosaccharides	*Bacteroides thetaiotaomicron VPI-5482*	GH105	52,65	AQKKKAVINDSNTPLHLLQPAYQGTYGDLTPEQVKKDID
17	Laminarinase	1,3-β-Glucanases	3.2.1.39	1,3-β-glucans such as laminarin	*Thermotoga maritima MSB8*	GH16	30,79	MMSRLVFALLLFPVFILAQNILGNASFDEPILIAGVDIDPP
18	GlcNAc-α-1,4-Gal-releasing endo-β-galactosidase	Galactosidases	3.2.1.-	GlcNAc-α-1,4-Gal-β-1,3-GalNAc-α1-Ser/Thr	*Clostridium perfringens ATCC 10543*	GH16	33,7	MFVFMLLLLLPFTISKAKDFPANPIEKAGYKLDFSDEFNG
19	Chitinase 1	Chitinases & Chitosanases	3.2.1.14	Chitin and chitosan	*Bacillus licheniformis DSM 13 = ATCC 14580*	GH18	49,24	MKIVLINKSKKFFVFSFIFVMMLSLSFVNGEVAKADSGKN
20	Endo-β-1,3-glucanase	1,3-β-Glucanases	3.2.1.39	Lichenan and laminarin	*Pyrococcus furiosus DSM 3638*	GH16	30,87	MKKEALLFLSLIFLVFVSGCIHHSTNQQLSSKQQVPEVIEID
21	Oligoalginate lyase	Alginate lyases	4.2.2.	Low-viscosity alginate	*Saccharophagus degradans 2-40*	PL17	81,37	MLSVNTIKNTLLAAVLVSVPATAQVSGNGHPNLIVTEQD
22	Xylanase D / Lichenase	1,3-1,4-β-Glucanases	3.2.1.73 3.2.1.8	1,3-1,4-β-glucans, in particular lichenan	*Ruminococcus flavefaciens 17*	GH16	30,59	MKKSIFKRYAAAVGLMASVLMFTAVPTTSNAADDQKTG

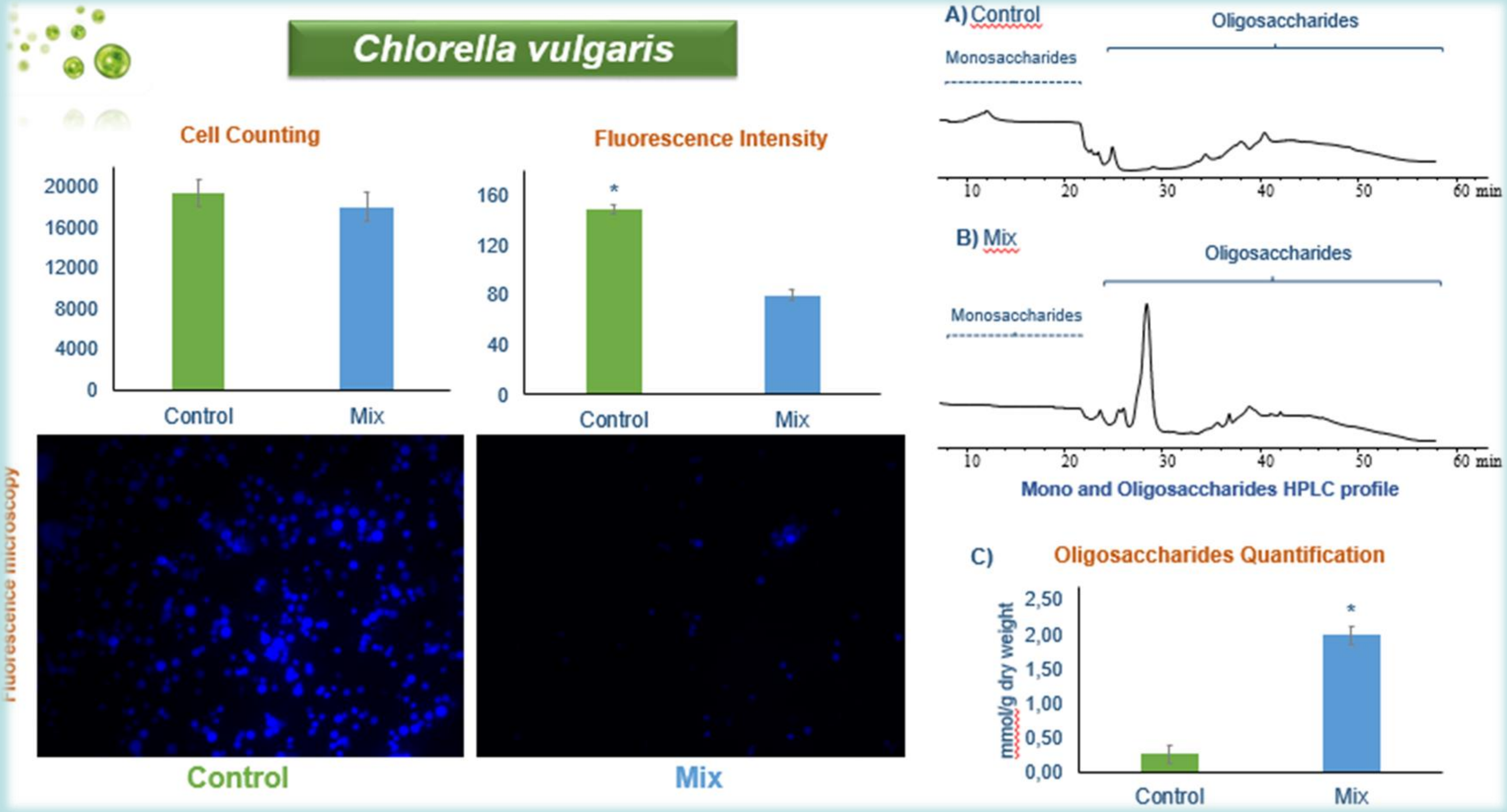
Development of an HTP approach to assess cell wall disruption by enzymatic digestion

Cloning, expression and production by an HTP platform



Development of an HTP approach to assess cell wall disruption by enzymatic digestion

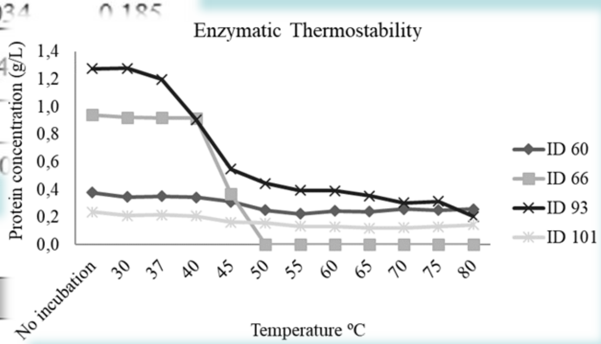
Individual and combined *in vitro* screening of enzymes



Development of an HTP approach to assess cell wall disruption by enzymatic digestion

Validation of the effective mix of enzymes

	Supernatant				Residue			
	Control	Mix	SEM	p-value	Control	Mix	SEM	p-value
Total proteins (mg/g microalgae)	14.6	341.2	17.01	< 0.001	776.4**	453.8**	17.03	< 0.001
Chlorophyll a (mg/g microalgae)	0.109*	0.116*	0.006	0.429	2.12**	2.86**	0.301	0.158
Chlorophyll b (mg/g microalgae)	0.154*	0.153*	0.010	0.948	1.27**	2.07**	0.388	0.217
Total chlorophylls (mg/g microalgae)	0.263*	0.269*	0.016	0.799	3.39**	4.93**	0.684	0.187
Total carotenoids (mg/g microalgae)	0.076*	0.083*	0.002	0.032	0.346**	0.268**	0.034	0.185
Total fatty acids (mg/g microalgae)	2.24	2.67	0.417	0.496	23.8	26.4	[illegible]	[illegible]
Fatty acid composition (% total fatty acids)								
14:0	1.81	1.21	0.216	0.097	1.37	1.30	[illegible]	[illegible]



Proteolysis resistance

ID	Time (min)				
	15	30	60	90	120
60	+	+	+	+	+
66	-	-	-	-	-
93	-	-	-	-	-
101	+	+	+	+	+

Enzyme mix selected for Spirulina degradation

Journal of
Animal Physiology and Animal Nutrition

A two-enzyme constituted mixture to improve the degradation of Arthrospira platensis microalga cell wall for monogastric diets

Journal:	*Journal of Animal Physiology and Animal Nutrition*
Manuscript ID	Draft
Manuscript Type:	Original Article
Date Submitted by the Author:	n/a
Complete List of Authors:	Coelho, Diogo; Universidade de Lisboa Faculdade de Medicina Veterinaria

Under minor revision, JAPAN

Mix	Lysozyme 104 + α-amylase (2) 72

Pr Patent nº20191000008190, INPI, PT

Enzyme mix selected for *Chlorella* degradation

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Novel combination of feed enzymes to improve the degradation of *Chlorella vulgaris* recalcitrant cell wall

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Coelho *et al.* 2019, Sci Rep 9 5382

Mix	Exo-β-glucosaminidase, Alginate lyase, Peptidoglycan N-acetylmuramic acid deacetylase and Lysozyme (CPE1314) (ID 60, 66, 93 and 101, respectively)

Pr Patent nº20181000067928, INPI, PT

Spirulina in broilers: experiment

Control diet (CON)

MA + 0.005% Rovabio® (MAR)

15% Spirulina (MA)

MA + 0.01% Lysozyme (MAL)

120 one-day broilers (*n*=30)

Experiment from d21 to d35

Spirulina in broilers: growth performance

Item	CON[1]	MA[1]	MAR[1]	MAL[1]	SEM	*P*-value
Body weight, g						
d 21	723	742	787	728	24.8	0.262
d 28	1,244	1,198	1,225	1,137	32.3	0.117
d 35	1,802[b]	1,697[ab]	1,717[ab]	1,613[a]	39.2	0.016
Body weight gain, g						
d 21-28	74.5[b]	65.2[a]	62.6[a]	58.5[a]	1.83	<0.001
d 28-35	93.0[b]	83.2[a]	82.0[a]	79.3[a]	2.25	<0.001
d 21-35	83.8[b]	74.2[a]	72.3[a]	68.9[a]	1.83	<0.001
Feed Intake, g						
d 21-28	107	105	107	97.9	3.09	0.126
d 28-35	141	138	138	132	3.3	0.342
d 21-35	124	121	122	115	3.0	0.191
Feed conversion ratio						
d 21-28	1.44[a]	1.60[b]	1.71[c]	1.68[bc]	0.022	<0.001
d 28-35	1.51[a]	1.66[b]	1.68[b]	1.67[b]	0.021	<0.001
d 21-35	1.48[a]	1.63[b]	1.70[b]	1.67[b]	0.017	<0.001
Relative weight of GI[2] tract, g/kg BW						
Crop	2.62	2.91	2.98	2.53	0.221	0.418
Gizzard	12.2	14.3	13.1	14.2	0.61	0.061
Liver	21.8	23.7	22.4	23.5	0.90	0.422
Pancreas	2.30	2.38	2.25	2.37	0.126	0.870
Duodenum	4.77	5.06	5.33	4.82	0.223	0.283
Jejunum	9.45	9.57	9.26	9.03	0.354	0.722
Ileum	8.29[ab]	8.19[ab]	7.39[a]	8.52[b]	0.281	0.042
Caecum[3]	3.74	3.79	4.68	3.63	0.361	0.164
Relative length of GI tract, cm/kg BW						
Duodenum	13.1[a]	15.7[b]	15.5[b]	14.8[b]	0.52	0.006
Jejunum	33.6[a]	37.8[b]	37.0[ab]	37.8[ab]	1.10	0.034
Ileum	37.2	38.7	38.8	40.7	1.15	0.224
Caecum	8.08	8.44	8.12	8.02	0.329	0.812
Content viscosity, cP						
Duodenum + jejunum	4.84[a]	7.08[ab]	7.32[ab]	9.20[b]	1.071	0.039
Ileum	9.19	10.8	11.8	10.8	0.962	0.306

Pestana *et al.*, Submitted

Spirulina decreased birds performance and increased some GI compartments and digesta viscosity

Lysozyme aggravated these MA effects on performance

Spirulina in broilers: health status

Item	CON[1]	MA[1]	MAR[1]	MAL[1]	SEM	*P*-value
Total lipids, mg/dL[2]	490[a]	588[d]	535[b]	556[c]	4.5	<0.001
Triacylglycerols, mg/dL	84.0[a]	142[c]	86.0[ab]	90.9[b]	1.42	<0.001
Total cholesterol, mg/dL	128[a]	148[b]	157[bc]	150[c]	2.2	<0.001
HDL-C, mg/dL[3]	107	105	104	110	1.8	0.130
LDL-C, mg/dL[3]	4.50[a]	14.1[b]	28.6[c]	29.3[c]	0.710	<0.001
VLDL-C, mg/dL[3]	16.8[a]	28.4[c]	17.2[ab]	18.2[b]	0.28	<0.001
Glucose, mg/dL	287[b]	265[a]	263[a]	273[ab]	5.4	0.017
Urea, mg/dL	1.45[ab]	1.10[a]	2.74[c]	1.68[b]	0.106	<0.001
Total protein, g/dL	2.73[a]	2.65[a]	3.11[b]	3.33[c]	0.032	<0.001
Creatinine, mg/dL	0.018[b]	0.006[a]	0.019[b]	0.005[a]	0.0010	<0.001
ALT[4], U/L	12.5	13.5	12.6	12.1	0.91	0.737
AST[5], U/L	255[b]	152[a]	585[c]	238[b]	15.9	<0.001
ALP[6], U/L	2821[c]	1760[b]	1063[a]	2956[c]	65.8	<0.001
γ-GT[7], U/L	15.5[ab]	20.7[c]	13.7[a]	17.2[b]	0.84	<0.001
TAC[8], μM (Trolox equiv.)	460	399	465	432	26.7	0.288

Pestana *et al.*, Submitted

Birds remained healthy during the entire trial

Spirulina in broilers: meat quality

	CON[1]	MA[1]	MAR[1]	MAL[1]	SEM	*P*-value	CON[1]	MA[1]	MAR[1]	MAL[1]	SEM	*P*-value
	Breast muscle						Thigh muscle					
pH24h	5.79	5.77	5.82	5.70	0.060	0.559	5.83	5.84	5.87	5.77	0.060	0.739
Lightness (L*)	49.2	46.6	47.6	46.9	0.84	0.139	49.2	49.0	48.0	47.4	0.47	0.051
Redness (a*)	4.53	5.31	5.50	5.37	0.288	0.089	8.25^{a}	9.49^{ab}	9.91^{b}	9.67^{ab}	0.430	0.046
Yellowness (b*)	4.38^{a}	10.7^{b}	11.7^{b}	12.3^{b}	0.546	<0.001	5.38^{a}	12.9^{b}	12.4^{b}	13.5^{b}	0.459	<0.001
Cooking loss, %	14.7	12.6	13.7	12.8	1.05	0.501	17.8	18.3	17.3	19.0	1.02	0.657
Shear force, kg	1.63	1.53	1.61	1.66	0.135	0.909	2.22	2.26	2.26	2.03	0.113	0.444
Diterpene profile, µg/g												
α-Tocopherol	4.03^{a}	2.18^{b}	2.18^{b}	2.35^{b}	0.187	<0.001	7.33^{a}	3.65^{b}	3.29^{b}	4.33^{b}	0.294	<0.001
γ-Tocopherol	0.618^{a}	0.270^{b}	0.279^{b}	0.288^{b}	0.0329	<0.001	0.658^{a}	0.345^{b}	0.308^{b}	0.379^{b}	0.0326	<0.001
α-Tocotrienol	nd	nd	nd	nd	-	-	0.313	0.249	0.260	0.301	0.0274	0.296
γ-Tocotrienol	nd	nd	nd	nd	-	-	0.291	0.247	0.297	0.298	0.0394	0.767
Pigments, µg/100 g												
Chlorophyll a[2]	8.78	10.6	10.7	9.53	1.491	0.774	4.90	10.1	9.75	8.36	1.905	0.219
Chlorophyll b[3]	13.7	16.6	17.7	15.5	2.50	0.703	8.51	13.8	12.8	10.8	2.974	0.608
Total chlorophylls[4]	22.4	27.2	28.4	25.1	3.89	0.716	13.4	23.9	22.6	19.2	4.721	0.414
Total carotenoids[5]	48.6^{a}	161^{b}	159^{b}	183^{b}	11.45	<0.001	48.8^{a}	153^{b}	158^{b}	186^{b}	9.46	<0.001
Total chlorophylls and carotenoids[6]	71.0^{a}	188^{b}	187^{b}	208^{b}	13.00	<0.001	62.2^{a}	177^{b}	181^{b}	208^{b}	10.70	<0.001

Pestana *et al.*, Submitted

Higher values of yellowness

Spirulina in broilers: conclusions

- Incorporation of 15% Spirulina decreased birds performance
- Mediated by increased digesta viscosity and lower protein digestibility
- Due to the resistance of MA liberated proteins to endogenous peptidases
- Lysozyme was effective in the degradation of SP cell wall and aggravated performance impairment of SP
- Commercial carbohydrate-degrading enzymes are not effective in the degradation of SP cell wall
- Meat quality and animal health was only slightly affected by SP, but not by enzymes
- Supplementation of diets receiving SP with lysozyme and a SP proteins-specific peptidase is a promising approach

Spirulina in piglets: experiment

Control diet (CON)

SP + 0.005% Rovabio® (SP+R)

10% Spirulina (SP)

SP + 0.01% Lysozyme (SP+L)

40 male post-weaned piglets (*n*=10)

Experiment lasted 28 days

Spirulina in piglets: growth performance

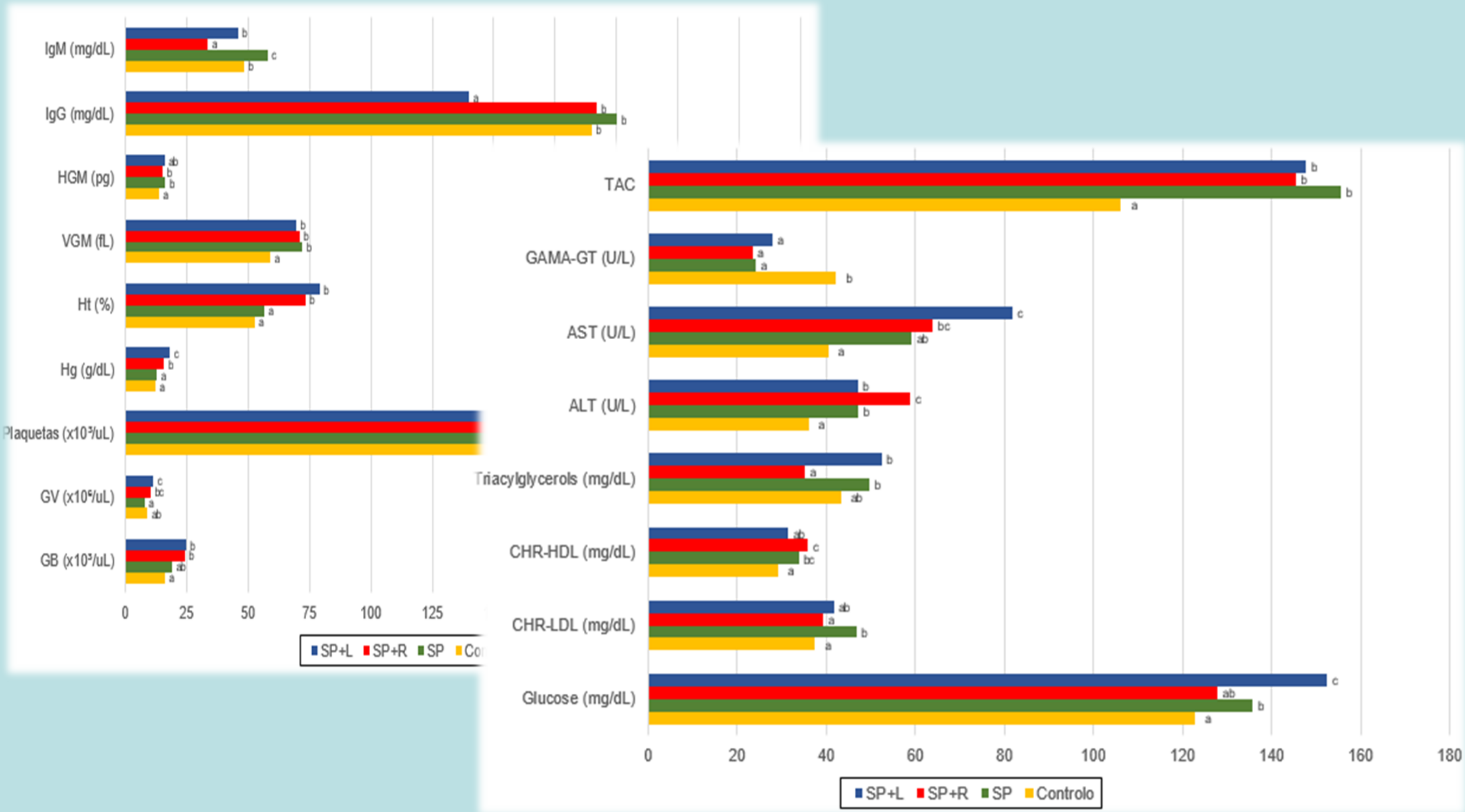
Item	Diets: Control	SP	SP+R	SP+L	SEM	*P* value
Live performance						
Initial weight (kg)	12.1	11.7	12.1	11.9	0.15	0.808
Final weight (kg)	31.0[a]	28.3[b]	28.4[b]	27.8[b]	0.40	0.009
ADFI (g)[1]	997	960	943	960	12.8	0.521
ADG (g)[2]	677[a]	593[b]	582[b]	567[b]	12.4	0.001
FCR[3]	1.48[a]	1.62[b]	1.62[b]	1.69[b]	0.023	<0.001
Faecal score[4]	0.070	0.223	0.145	0.198	0.032	0.355
TTAD (%)[5]						
DM	79.6[a]	77.6[b]	77.3[b]	79.5[a]	0.35	0.014
OM	83.1[a]	81.3[bc]	81.1[c]	82.9[ab]	0.32	0.031
Ash	42.3[a]	34.4[b]	34.1[b]	43.8[a]	1.14	<0.001
CP	80.6[a]	73.2[b]	73.4[b]	75.4[b]	0.81	<0.001
CF	55.6[a]	57.8[ab]	60.4[bc]	62.8[c]	0.78	<0.001
NDF	39.8	39.0	37.5	45.4	1.17	0.071
ADF	23.0[a]	28.9[ab]	31.1[ab]	37.3[b]	1.86	0.039
Relative length of gastrointestinal tract (m/kg)						
Small intestine	0.466[a]	0.487[ab]	0.532[bc]	0.541[c]	0.0101	0.007
Large intestine	0.110	0.122	0.128	0.121	0.0032	0.154
Content viscosity (cP)						
Duodenum + jejunum	3.16[a]	4.96[b]	5.32[b]	6.11[b]	0.320	0.005
Ileum	5.88[a]	8.97[b]	7.77[ab]	8.63[b]	0.403	0.023

Martins *et al.*, Submitted

Spirulina impaired growth performance, protein digestibility and digesta viscosity

Lysozyme improved fibre digestibility and aggravated performance effects of SP

Spirulina in piglets: health status



Martins *et al.*, Submitted

Piglets remained healthy during the entire trial

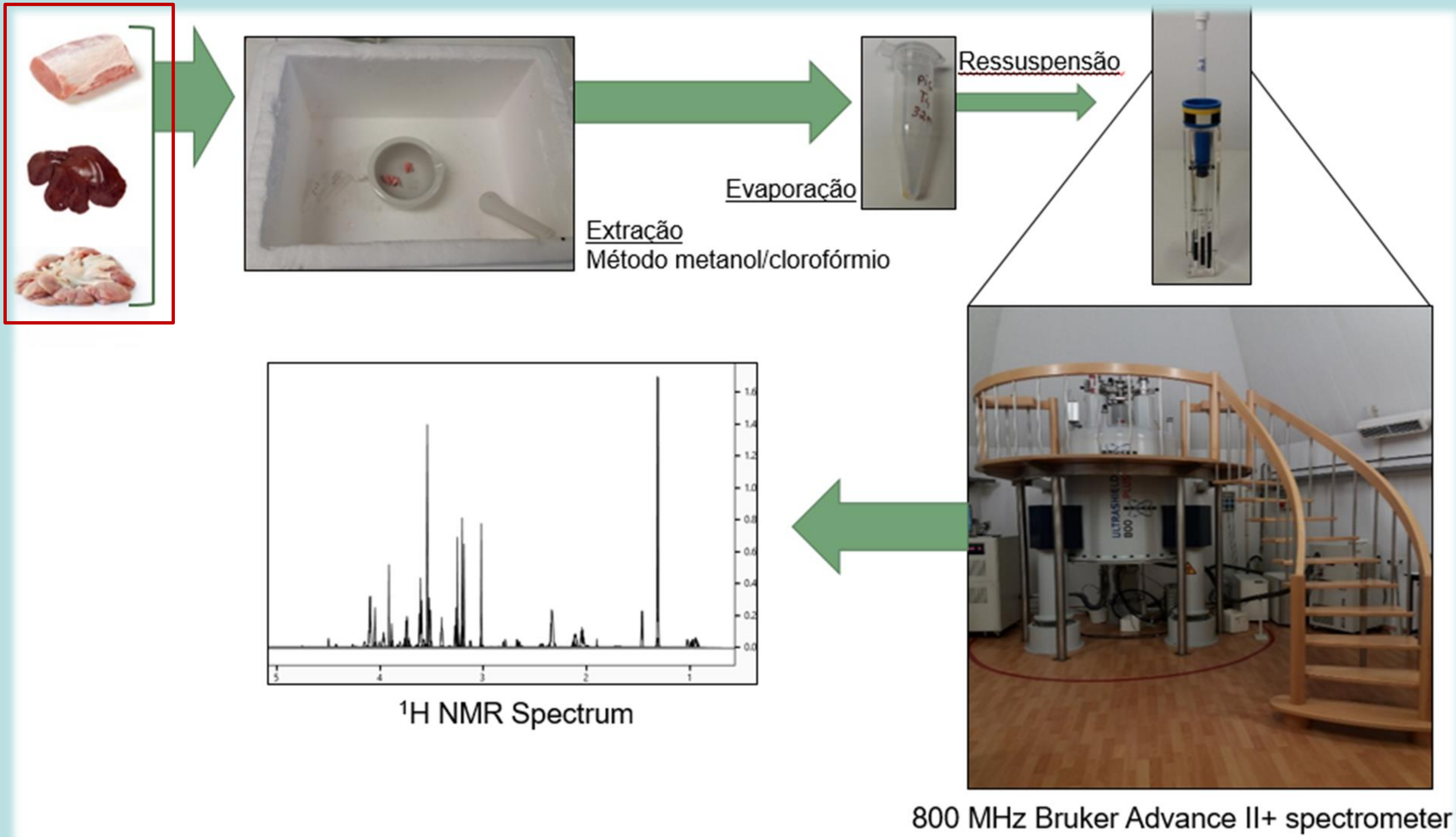
Spirulina in piglets: meat quality

Item	Diets				SEM	*P* value
	Control	SP	SP+R	SP+L		
pH 24 h	5.55	5.50	5.52	5.55	0.062	0.902
Colour						
L*	50.4^{a}	51.5^{ab}	53.3^{b}	51.9^{ab}	0.70	0.022
a*	7.08^{a}	7.68^{ab}	6.99^{ab}	8.17^{b}	0.263	0.008
b*	0.93^{a}	1.95^{ab}	2.29^{b}	2.38^{b}	0.264	0.001
TBARS[1]						
Day 0	0.233	0.233	0.224	0.245	0.0109	0.588
Day 3	0.350^{a}	0.814^{b}	0.363^{ab}	0.546^{ab}	0.1237	0.041
Day 7	0.590	1.968	1.088	2.091	0.4292	0.078
Pigments						
Chlorophyll-a[1]	0.221^{a}	0.346^{ab}	0.291^{ab}	0.397^{b}	0.0447	0.045
Chlorophyll-b[2]	0.328^{a}	0.508^{ab}	0.475^{ab}	0.653^{b}	0.0762	0.035
Total chlorophylls[3]	0.549^{a}	0.853^{ab}	0.766^{ab}	1.051^{b}	0.1196	0.037
Total carotenoids[4]	0.092^{a}	0.146^{b}	0.150^{b}	0.153^{b}	0.0143	0.013
Total chlorophylls and total carotenoids[5]	0.641^{a}	0.999^{ab}	0.916^{ab}	1.204^{b}	0.1316	0.033
Diterpene profile						
β-Carotene	0.081	0.081	0.077	0.082	0.0034	0.668
α-Tocopherol	0.498	0.389	0.504	0.366	0.0479	0.090
γ-Tocopherol	0.030	0.030	0.031	0.027	0.0021	0.522

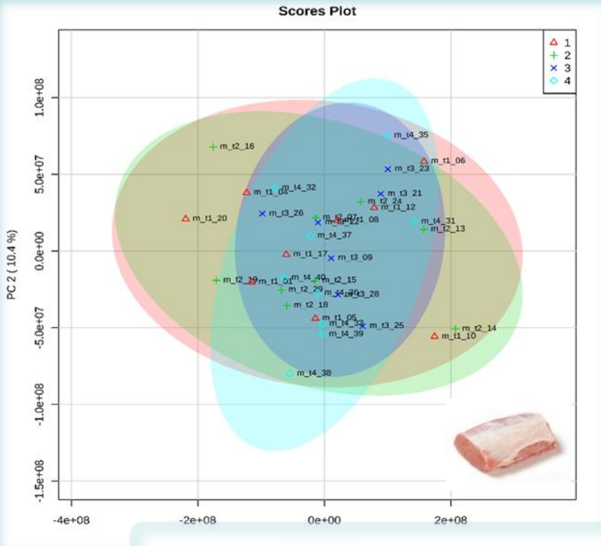
Martins *et al.*, Submitted

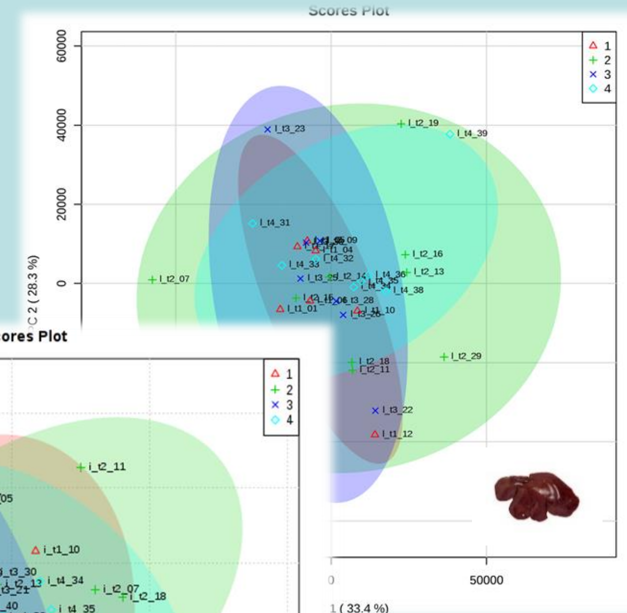
Spirulina and enzymes did not affect meat quality

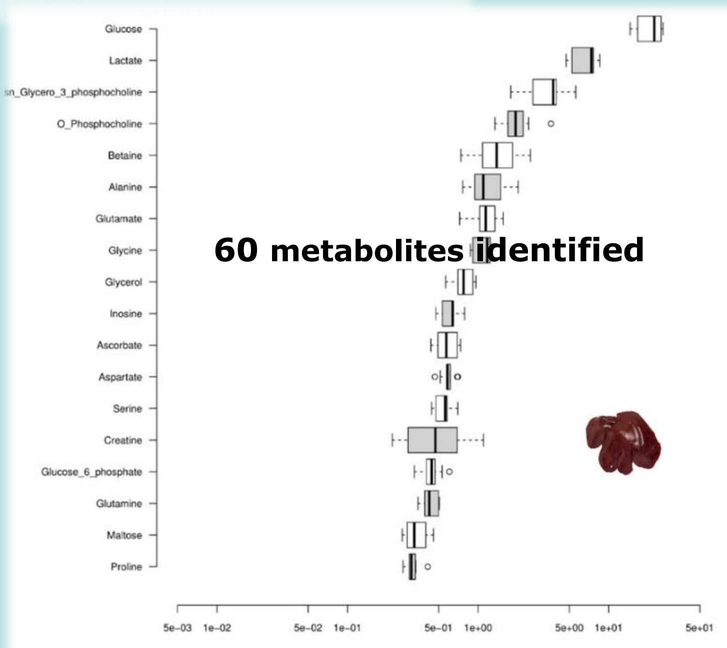
Spirulina in piglets: metabolomic analysis



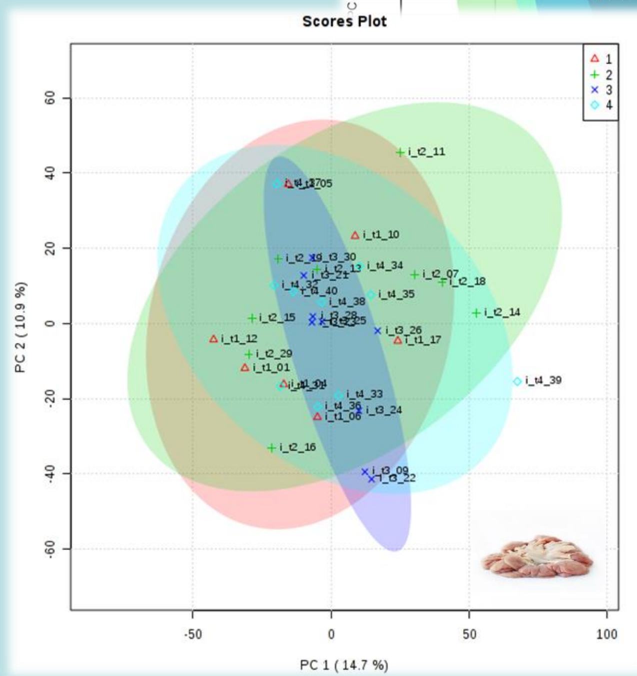
Spirulina in piglets: metabolomic results







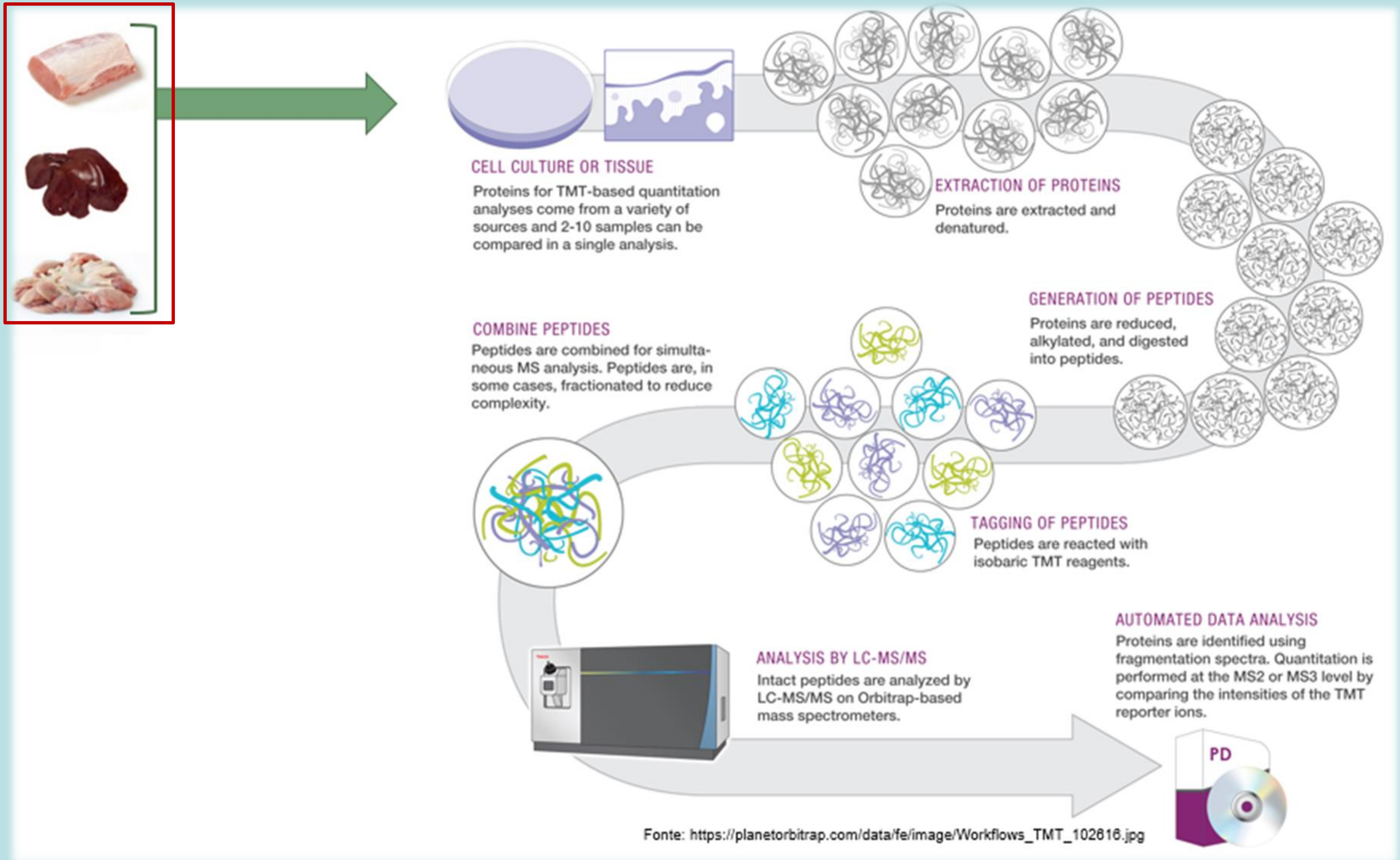
60 metabolites identified



Martins *et al.*, Unpublished

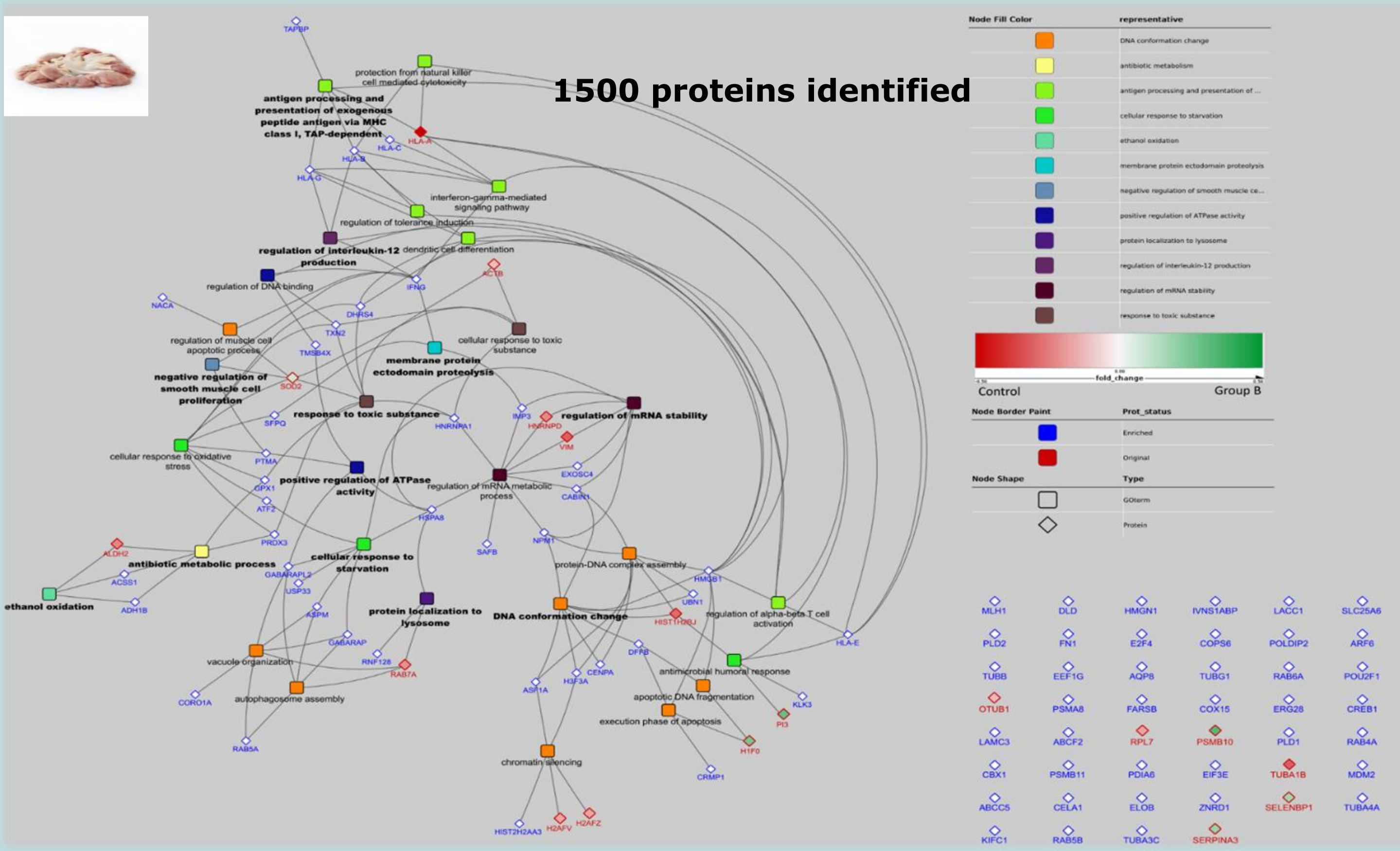
PCA analysis did not show differences among diets

Spirulina in piglets: proteomic analysis



Spirulina in piglets: proteomic results

1500 proteins identified



Martins *et al.*, Unpublished

<u>Liver</u>: SP increased catabolism of carbohydrates and fatty acid oxydation

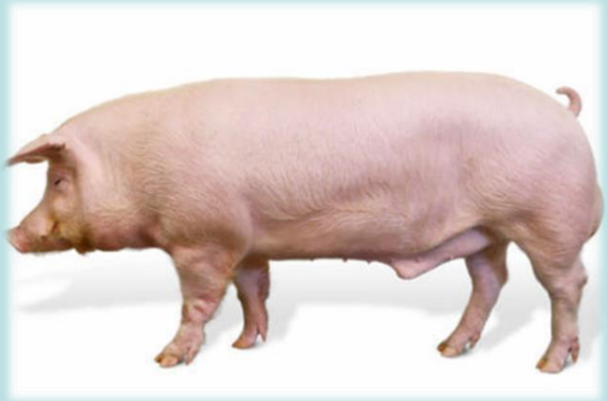
<u>Gut</u>: SP improved imune status and oxydative stress

Spirulina in piglets: conclusions

- Incorporation of 10% Spirulina impared piglets performance
- Mediated by increased digesta viscosity and lower protein digestibility
- Due to the resistance of microalga liberated proteins to endogenous peptidases
- The assessment of exogenous peptidases are ongoing
- Lysozyme was effective in the degradation of SP cell wall
- Commercial carbohydrate-degrading enzymes are not effective in the degradation of SP cell wall
- Meat quality and animal health was not affected by SP or enzymes
- SP improved gut imune status and oxydative stress

Similar *Chlorella* trials are ongoing

- **Poultry experiment** (trial done, samples under analysis)
- **Piglets digestibility experiment** (samples under analysis)
- **Piglets performance experiment** (will start in December)
- **Finishig pigs experiment in a farm** (trial ongoing)
- **Finishing pigs experiment** (will start in September)



General conclusions and challenges

- MA have interesting properties for animal feeding:

Chemical composition is similar to conventional crops

Could improve meat quality

Could help to balance food-feed-biofuel industries

Is a sustainable marine resource

- However, their use in animal feeding is currently limited

- Challenge of the nutrients bioavailability (monogastrics):

Strategies to valorize their nutritional value are needed

CAZymes could help in the increase of nutrientes bioavailability

MA proteins are resistant to endogenous peptidases

The use of exogenous peptidases seems to be necessary

- Challenge of the cost-effective use by the feed industry:

MA cultivation technology is inefficient

Reduction of production costs

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