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In vitro gas production of 8 selected seaweeds as indicator for rate and extent of rumen fermentation

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Enlargement of feed resources

Identification of novel feeds
Development of new additives

Land degradation

Food-fuel-feed competition

↓

- By-products from food industry
- Single-cell protein (bacteria, yeast, fungi, microalgae)
- Plant extracts
- Aquatic biomass (seaweed, duckweed)
- …

CH_4 emissions

Climate change

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Phaeophyceae

- Shallow waters or on shoreline rocks
- Very flexible steams
- Can reach large size
- Easy to harvest
- Low – medium CP content
- Rich in minerals
- Cell wall: cellulose, alginic acid and fucoidans
- Reserve carbohydrate: laminarin
- Rich in iodine

Rhodophyceae

- Bright pink colour caused by biloprotein pigments
- From low tide marks to 100 m depth
- Some of them largely used as food (e.g. nori for sushi)
- Rich in protein
- Cell wall: carragenans and agars
- Reserve polysaccharide: floridean starch
- Limited amount of iodine

Clorophyceae

- Green colour due to chlorophyll
- Shallow waters and tide pools (abundant light)
- Up to 45 cm
- Fast growing
- Good protein content
- Low energy content
- High insoluble dietary fibre (glucans)

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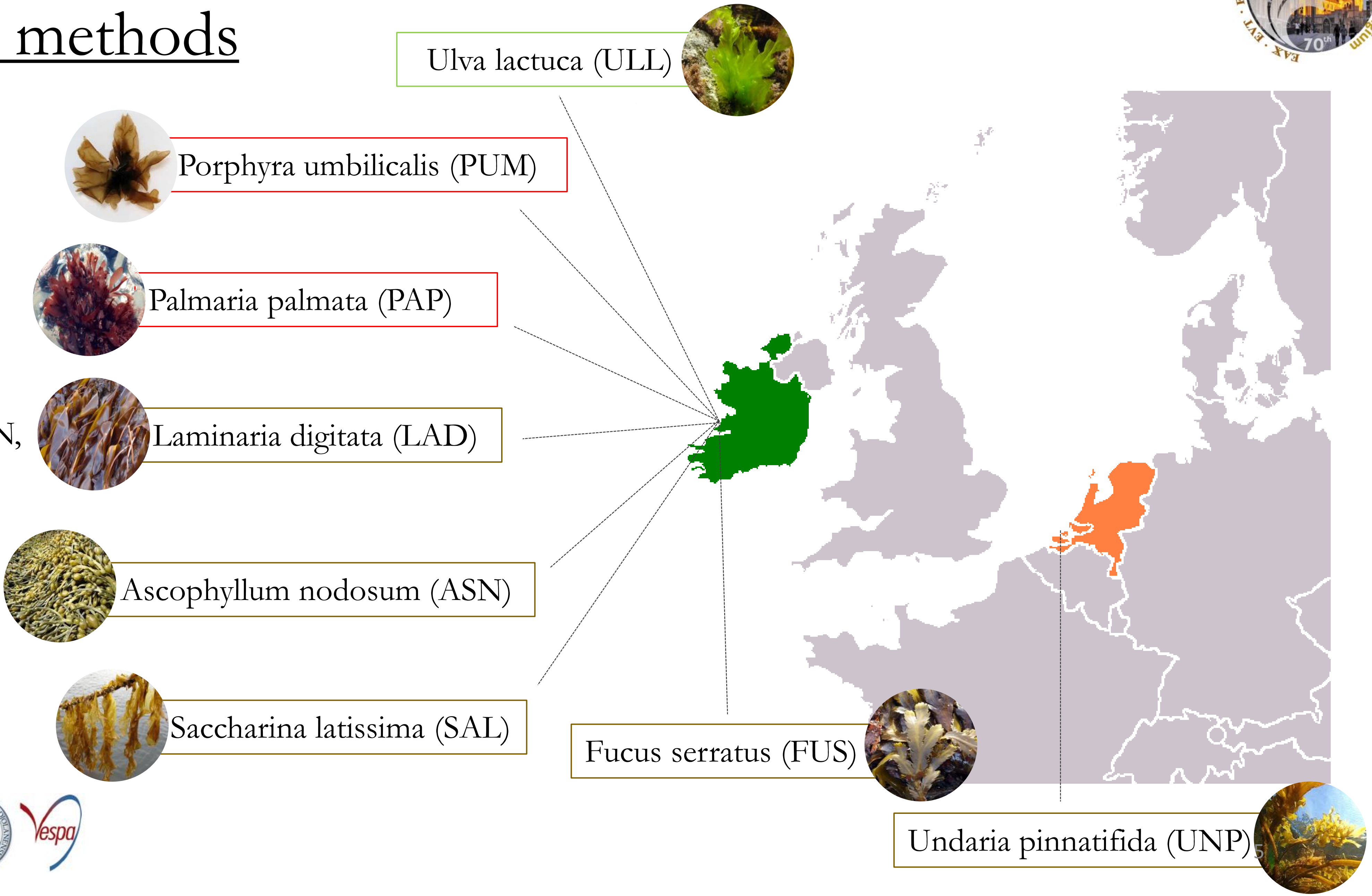
Aim of the experiment

Screening evaluation of 8 North West European seaweed species as alternative feed for dairy cattle, according to *in vitro* total gas production (TGP) and CH_4 production

Materials and methods

Seaweeds

- Collection season:
  - Spring 2018 (UNP, LAD, SAL, PUM)
  - Autumn 2018 (ASN, FUS, ULL, PAM)
- Wild
- Drying method: dehumidifier (<70°C)



In vitro test – total gas and methane production

Rumen fluid from 2 different lactating Holstein-Friesian cows
(fed grass and corn silage diet)
+
Buffer solution
+
Sample (0,5 g)

- Each sample in triplicate
- Control: grass silage

72 h in shaking water baths at 39 °C, with individual bottles connected to an automated gas production measuring system

Gas samples (10 μL) at 0, 2, 4, 6, 8, 10, 12, 24, 30, 36, 48, 56, 72 h from the head space of the fermentation bottles using a gas-tight syringe and analysed for CH_4 by gas chromatography

Pellikaan et al., 2011

All seaweeds were analysed for: DM, Ash, CP, EE, NDF, starch, iodine and heavy metal content, total phenolic content, fatty acids composition

Statistical analysis

Cumulative gas and CH_4 production curves were fitted with a monophasic Michaelis–Menten equation *(Groot et al., 1996)*

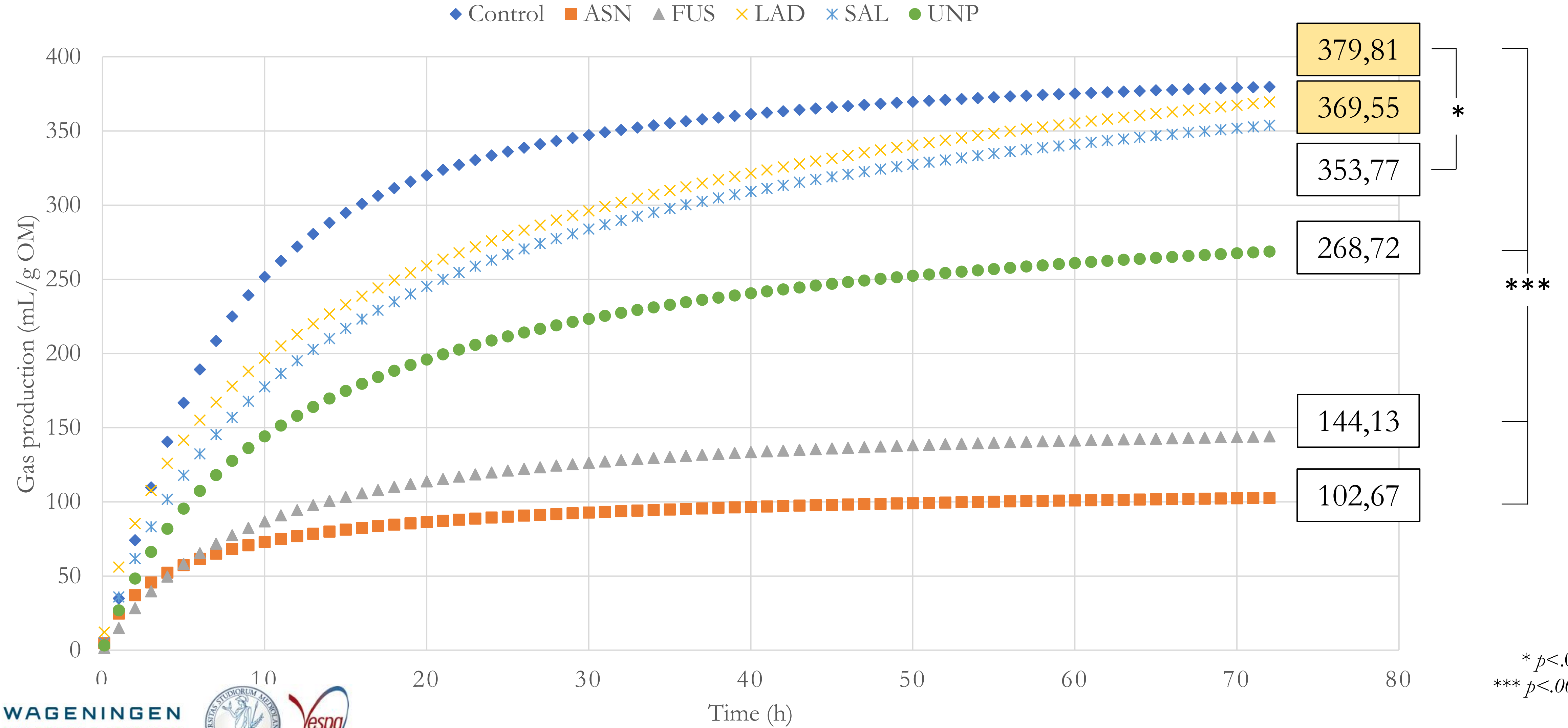
$$OMCV = \sum_{i=1}^{n} \frac{A_i}{1 + (B_i/t)^{C_i}}$$

Where:
OMCV = gas or CH_4 production (ml/g of incubated OM);
A_i = asymptotic gas production (ml/g of incubated OM);
B_i = time at which half of the asymptotic gas or CH_4 production has been reached ($t_{1/2}$, h);
C_i = the sharpness of the switching characteristics of the profile;
t = time (h)

Total gas production and CH_4 production at 72 h were analysed using a GLM procedure in SAS 9.2

Results

Total gas production – brown species

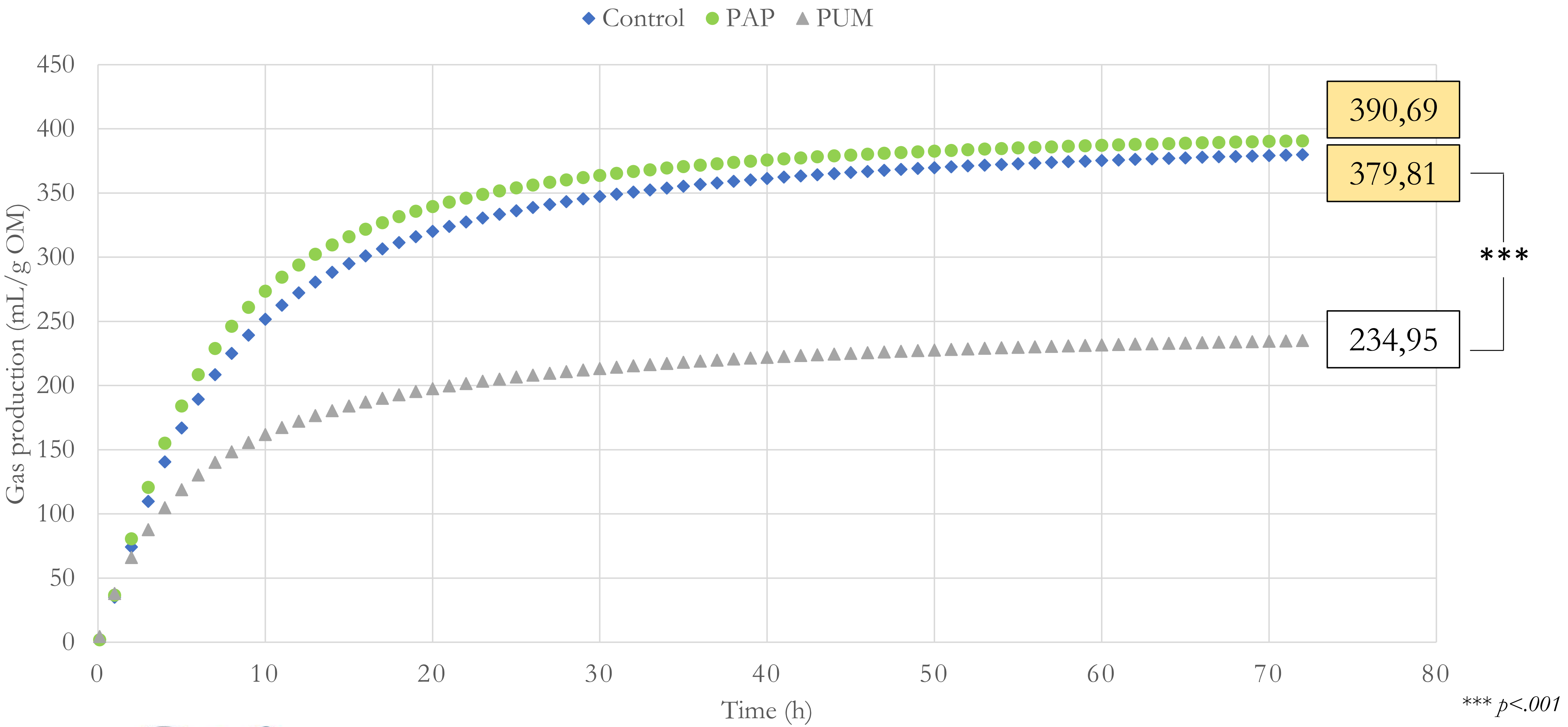


* $p<.05$
*** $p<.001$

		***	***		*	***
		A. nodosum	***F. serratus***	***L. digitata***	***S. latissima***	***U. pinnatifida***
DM (g/kg)	g/kg	890.9	877.3	881.5	893.3	917.7
ASH	g/kg DM	187.3	207.3	231.5	299.1	385.5
NDF		168.6	162.1	132.2	161.2	155.1
Crude Fibre		61.6	58.1	63.8	76.2	49.0
Crude Fat		34.9	32.7	7.4	4.3	5.6
STARCH		3.5	6.6	1.1	1.9	1.3
N		10.6	11.4	15.3	25.9	42.1
Protein (N*6.25)		66.4	71.3	95.7	162.0	263.3
Protein (N*4.75)		50.4	54.2	72.7	123.2	200.1
I	g/kg DM	984.7	2570.0	58.2	45.8	26.6
Cu		<5	<5	<5	5.9	5.8
As		34.2	94.8	6.6	9.2	33.3
Cd		0.2	0.1	0.3	0.2	0.2
Saturated FA	g/100g	42.0	30.0	12.0	45.0	67.0
Unsaturated FA		187.0	74.0	4.0	26.0	123.0
Monounsaturated FA		111.0	23.0	3.0	16.0	23.0
PUFA		76.0	51.0	1.0	10.0	100.0
Total phenolic content	GA/g	28.68	18.82	0.47	0.69	1.27

Muizelaar et al., not published

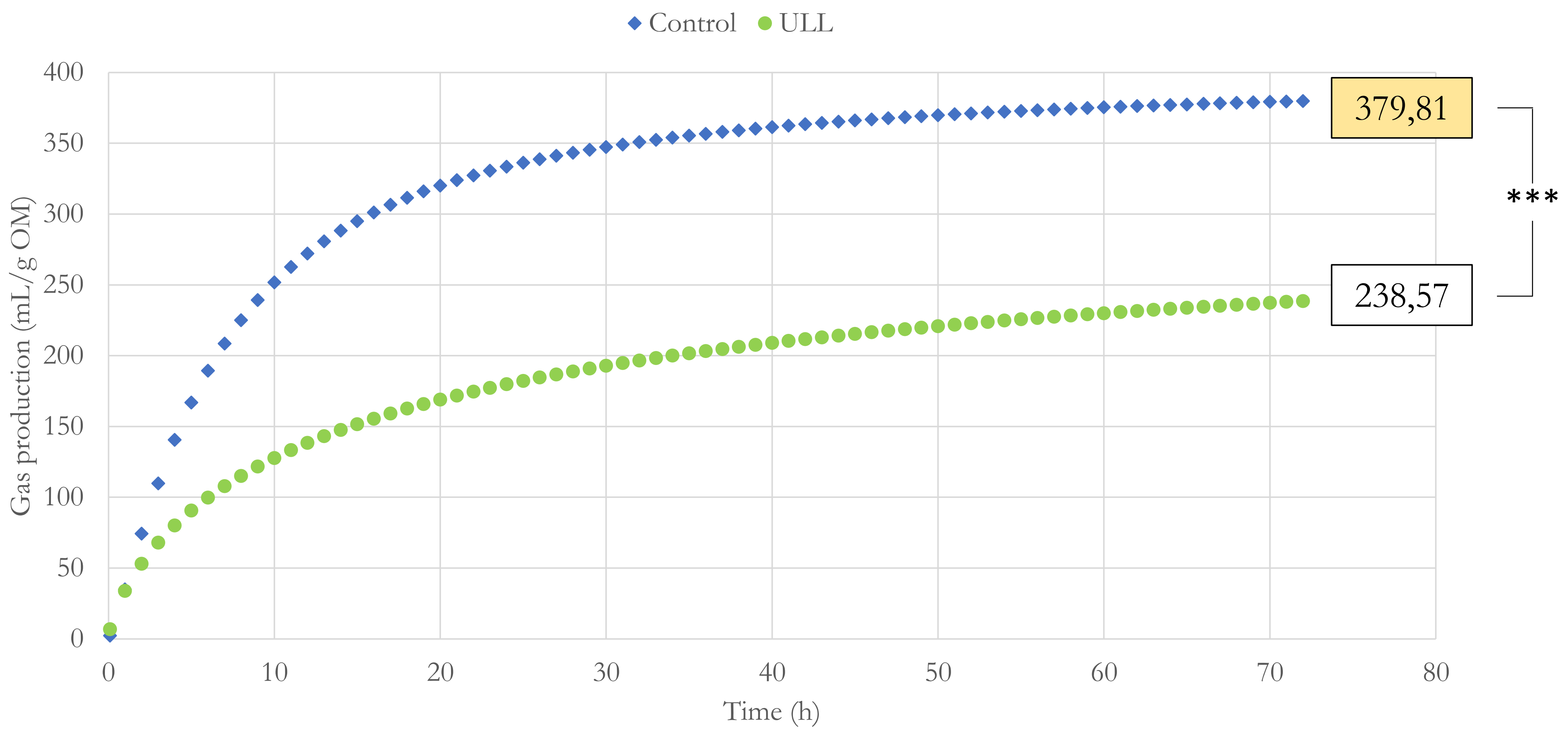
Total gas production – red species



			***
		P. palmata	***P. umbilicalis***
DM (g/kg)	g/kg	926.5	861.4
ASH	g/kg DM	302.6	196.3
NDF		252.9	302.6
Crude Fibre		36.7	27.9
Crude Fat		7.9	3.4
STARCH		16.0	89.6
N		31.5	36.0
Protein (N*6.25)		196.8	225.0
Protein (N*4.75)		149.5	171.0
I	g/kg DM	181.7	65.4
Cu		<5	34.2
As		11.7	12.1
Cd		0.1	0.0
Saturated FA	g/100g	53.0	60.0
Unsaturated FA		108.0	21.0
Monounsaturated FA		11.0	14.0
PUFA		97.0	7.0
Total phenolic content	GA/g	1.67	3.36

Muizelaar et al., not published

Total gas production – green species

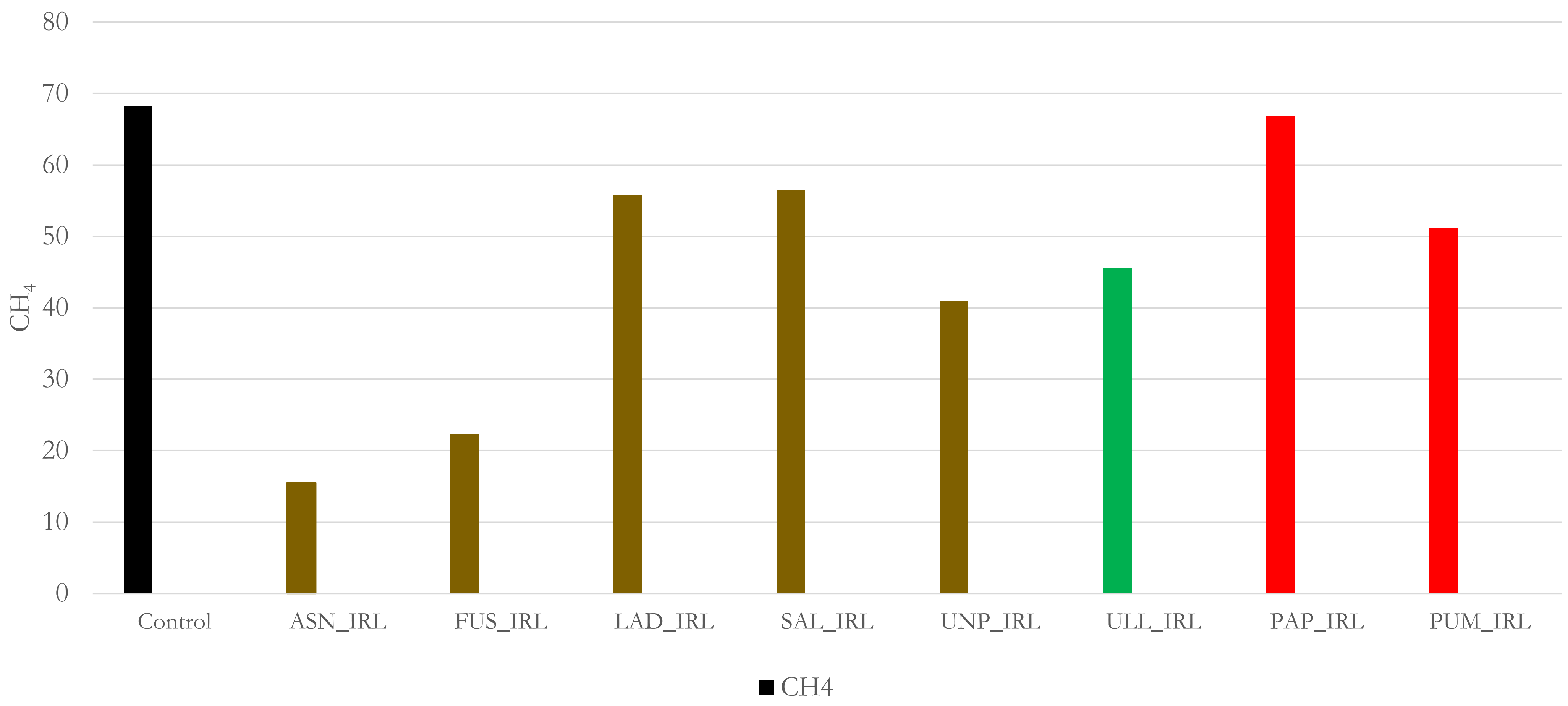


*** *p<.001*

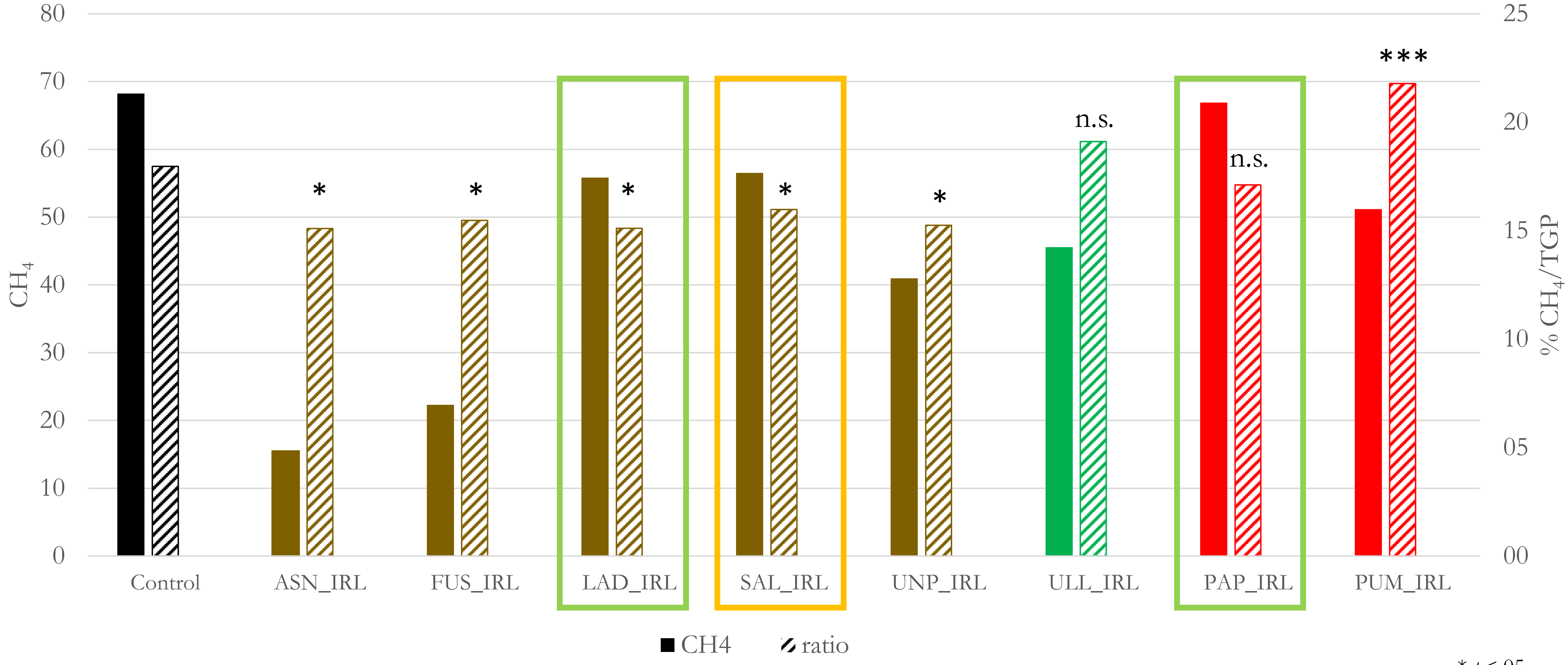
		*** *U. lactuca*
DM (g/kg)	g/kg	846.2
ASH	g/kg DM	387.7
NDF		237.5
Crude Fibre		70.6
Crude Fat		3.5
STARCH		25.9
N		21.1
Protein (N*6.25)		132.1
Protein (N*4.75)		100.4
I	g/kg DM	2676.7
Cu		<5
As		15.1
Cd		0.6
Saturated FA	g/100g	57.0
Unsaturated FA		14.0
Monounsaturated FA		9.0
PUFA		5.0
Total phenolic content	GA/g	1.16

Muizelaar et al.,
not published

CH_4 total production and CH_4 % per TGP



CH_4 total production and CH_4 % per TGP



* $p<.05$
*** $p<.001$

Conclusion and future perspective

- According to TGP and CH_4 production, *P. palmata*, *S. latissima* and *L. digitata* might be considered as potential (partial) substitute of grass silage
- Further investigation are required to assess their validity as potential replacer (OM degradability, VFA, amino acid composition, digestibility, …)
- Additional test with rumen fluid form cows familiar with seaweeds
- Evaluation of potential effect on animal health
- Evaluation of sustainability of their cultivation

Thanks for your attention

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Supplementary 1 – Polyphenols in brown seaweeds

Species	**Collecting location**	**Extraction method (% MeOH)**	**Total phenolic content (mg GAE/g)**
Ascophyllum nodosum	Ireland	60%	4.5 *
Fucus serratu			
Laminaria di			
Laminaria di			
Saccharina la			
Saccharina la			
Undaria pinn			
Ascophyllum			
Fucus serratu			
Laminaria digitata	Ireland	75%	0.47
Saccharina latissima	Ireland	75%	0.69
Undaria pinnatifida	Netherlands	75%	1.27

Research Article

SCI

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Laminaria digitata phlorotannins decrease protein degradation and methanogenesis during *in vitro* ruminal fermentation

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*mg GAE/g dw

erali´c Mekini´cg et al., 2019

Muizelaar et al., not published

Supplementary 2 – Arsenic in seaweeds

Table 1
Total arsenic (As_T) and inorganic arsenic (As_I) contents (mg kg^{-1} DW) and As_I/As_T ratios in red (Rhodophyta), brown (Phaeophyta) and green algae (Chlorophyta).

Phylum		As_T	As_I	As_I/As_T (%)
Rhodophyta	Range	0.13–50	0.048–3.0	0.2–61.54
	Average	13.71 ± 9.79^a (n = 92)	0.36 ± 0.32^a (n = 44)	5.97 ± 12.71^a (n = 44)
Phaeophyta	Range	1.89–245.19	0.04–115.56	0.08–85.29
	Average	50.36 ± 44.60^b (n = 154)	16.65 ± 29.32^b (n = 65)	21.59 ± 28.54^b (n = 65)
Chlorophyta	Range	0.59–28.53	0.02–0.40	0.48–16.09
	Average	5.59 ± 5.08^a (n = 36)	0.20 ± 0.13^a (n = 14)	5.28 ± 5.78^a (n = 14)

** Data shown are the means ± standard deviations from data collected from published studies available up to June 2017. Letters in superscript indicate significant differences between groups at the $P \leq .05$ level.

Ma et al., 2018

Table 2
Seaweed species containing high level of total arsenic (As_T).

Species	Phylum	Zone	*As_T	Methodology	Reference
Fucus vesiculosis	Phaeophyta	UK	140	HPLC-ICP-MS	Pedersen and Francesconi, 2000
Laminaria spp.	Phaeophyta	Brittany	134	HPLC-ICP-MS	McSheehy and Szpunar, 2000
L. digitata	Phaeophyta	France	126 ± 5	HPLC-ICP-MS	Garcia-Salgado et al., 2012
L. digitata	Phaeophyta	USA	106.73	ICP-MS	Taylor and Jackson, 2016

Ma et al., 2018

Supplementary 3 – CP comparison

	CP (g/kg DM)	
	N*6,25	N*4,75
Grass silage (control)	150,4	
L. digitata	95,7	72,7
P. palmata	196,8	149,5
S. latissima	162.0	123,2

Muizelaar et al., not published