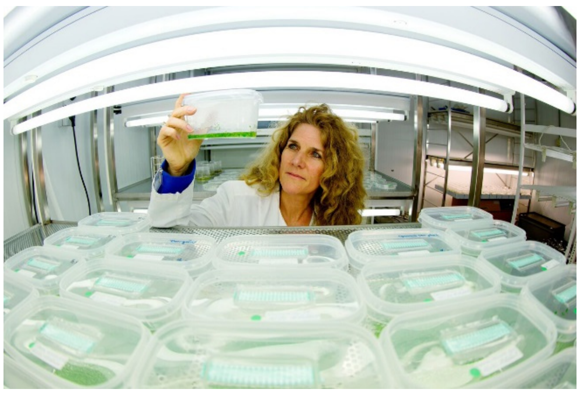
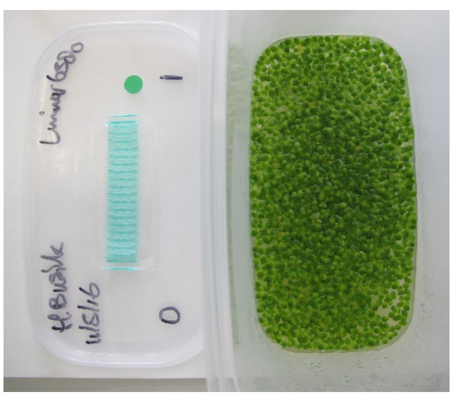
GADING FINAL REPORT

WUR-Duckweed expertise and analysis within GADING project





Wageningen University & Research

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GENERAL INTRODUCTION

The Indonesia Domestic Biogas Programme, branded in Indonesia as the BIRU programme (see www.biru.or.id), has since 2009 successfully stimulated the Indonesian biogas sector, which to date has led to the construction of more than 14,000 household bio-digesters. This multi-actor programme, undertaken in close cooperation with the Indonesian Ministry of Energy and Mineral Resources (MEMR), works also with many other actors (including the private sector, for example Nestle, Rabo Bank, Bank Syariah Mandiri) to reach a range of outputs, including development of entrepreneurial and technical skills, the establishment of small companies (construction of digesters and manufacture of biogas appliances), the development of sustainable agriculture by processing and applying the bio-digestate (bio-slurry) on the fields to enhance yields, access to micro-finance, etc.

The local demands of the farmers has changed and they now request new high value protein sources. Discussions with various stakeholders, including Wageningen University & Research (WUR), has led to the development of the concept to assess whether duckweed can become a profitable and safe high protein fodder for cattle (fattening and dairy), chicken, duck and fish production, while applying cow dung based on high protein feeding as digester feed stock to create high quality organic fertilizer.

In this project we analysed possibilities to mitigate waste streams (bio-slurry from bio-digesters) at the farm level whilst introducing new income sources from integrated farming. Food, feed and energy production are integrated in sustainable duckweed (Lemna) – animal farming in such a way that waste streams are reduced. In this system farmers, but also other sectors, will be less dependent on external resource supply using the cradle to cradle concept.

To analyse possibilities to integrate Lemna farming in traditional small scale farming in Indonesia the following actions were undertaken by WUR:

1. Analyse potential productivity of duckweed under laboratory conditions when grown on a commercial fertilizer
2. Derive harvesting strategies based on point 1 and selection of most promising species/strain for outdoor experiments
3. Analyse productivity in The Netherlands in 6 m^2 outdoor ponds for commercial fertilizer grown and bio-slurry grown duckweed
4. Analyse nutritional value/ nutrients composition of duckweed (Lemna minor) (point 1)
5. Analyse duckweed biomass grown under several outdoor conditions (point 3).
6. Analyse nutrients composition and heavy metals of duckweed cultivated in Indonesia at different pilot plants
7. Digestibility of Lemna grown under different conditions (point 3)
8. Advice on bio-slurry application rates and harvesting schemes for small scale farmers in Indonesia based on experimental results at WUR and farm visits
9. Economic analyses of integrated duckweed – animal small scale farming systems

All these actions fit in the four research themes: Analysis bioslurry i.r.t. Lemna feeding; Lemna production & bioslurry; Biochemical analysis & Lemna growth; Economic potential Lemna in Indonesia.

II METHODS

Environmental conditions growth room

Plants were grown in a growth room with the following conditions: average photosynthetic flux density ~325 µmol m^{-2} s^{-1} for a 16 h light period. Light was provided by fluorescent tubes and incandescent lamps. Day temperature was set at 23°C and night temperature at 20°C, resulting in water temperatures varying between 22-26°C (adapted from Van der Werf et al. 1993).

Growth analysis

Plants were grown in 40 L containers with a surface area of 2100 cm^2 containing a non-limiting nutrient solution. CO_2 concentration in the growth room was maintained at ambient (400-450ppm). Each container was divided into 15 compartments using a plexi glass construction (Fig. 1). Every four and three days two randomly chosen compartment per species and per container (n=6), were destructively harvested and dry weight was determined on (38 °C) oven-dried plant material. Table 1 gives the final adjusted concentrations in the medium after testing compared to the original medium used in the preliminary experiments (see report Q2 for more details).

Table 1. Nutrient concentrations used for growth analyses of eight Lemna species in Q3 (New) compared to the ones used in Q2 (Old).

Macro-nutrients	New	Old	unit
KH_2PO_4	106	57	mg/l
KNO_3	404	202	mg/l
$MgSO_4.7H_2O$	136	52	mg/l
Fe- EDTA	0.048	0.036	**mM**
$Ca(NO_3)_2.4H_2O$		80	mg/l
$CaCl_2.2H_2O$	66		mg/l
Micro-nutrients			
$MnSO_4.H_2O$	2.200	0.608	mg/l
H_3BO_3	2.970	0.817	mg/l
$ZnSO_4.7H_2O$	0.550	0.152	mg/l
$CuSO_4.5H_2O$	0.086	0.025	mg/l
$Na_2MoO_4.2H_2O$	0.139	0.039	mg/l

Fig. 1 Experimental set-up indoor laboratory experiments

Duckweed strains (*Lemna minor*) were grown in the growth chamber.

Duckweed grown on commercial fertilizer versus bio-slurry

In the summer of 2016 we started an outdoor experiment in 6 m^2 ponds comparing Lemna grown on nutrient solution versus Lemna grown on bio-slurry. For the commercial fertilizer the same nutrient concentration was used as the one in the laboratory experiments. Based on an initial concentration of nitrogen a starting mixing ratio of 1.4% of the pond volume was chosen, resulting in an ammonium-N concentration of 1.4 mM (and total N of 4 mM which is similar to the total amount of nitrogen in the commercial fertilizer, see table 1). At regular intervals the ammonium concentration was analysed for the bio-slurry treatment and a bio-slurry amount was added such that a target concentration of 1.4 mM ammonium-N would be reached. Similarly, commercial fertilizer concentration was analyzed at the same time, and an amount of nutrients were added, such that the initial concentration would be reached (see table 1). Over the experimental period bio-slurry mixing ratios of 0.3-0.8% were applied. Each week 40% of the surface area was harvested for both treatments. Over time standing crop before and after harvest remained fairly constant (data not shown). The experiment ran until growth ceases due to low temperatures (Oct-Nov). Nergena: growth on mineral-based nutrients and Lelystad: growth on digest-based nutrients. Four harvests: 28-07, 25-08, 22-09 and 19-10-2016 and each three biological repetitions (culture baths).

Biochemical analyses

Samples were either freeze-dried or dried in oven at 40°C and LCMS-profiling was performed of semi-polar extracts to determine global differences in metabolome. Dried or freeze-dried plant material was used for the analyses. For the biochemical analysis several mass-spectrometry coupled to liquid- or gas chromatography methods were used as shown in figure 2. Dried plant material was used for nutritional- and biochemical analyses. For nutritional analysis several main food nutrients were analysed such as: protein, starch, dietary fibres, fat and carbohydrates, plus crude ash content. For the samples sent from Indonesia one sample per location/ drying method (the last sample of each harvesting series) was analysed for amino acid profile. Total amino acid profile was analysed since proteins were hydrolysed in these samples and three samples of each series were mixed equally and analysed for heavy metals (Arsenic, Cadmium, Mercury, Lead) and minerals (Sodium, Magnesium, Phosphorus, Potassium, Calcium, Manganese, Iron, Copper, Zinc).

To analyse overall differences between samples, a statistical method was used, Principal Component Analysis (PCA; https://en.wikipedia.org/wiki/Principal_component_analysis). This statistical method visualizes the difference of samples in a 2D or 3D graph based on a data set. In this study the metabolomics data (all metabolites that could be detected) per sample are used to plot the samples in a 2D or 3D axis showing how correlated the samples are.

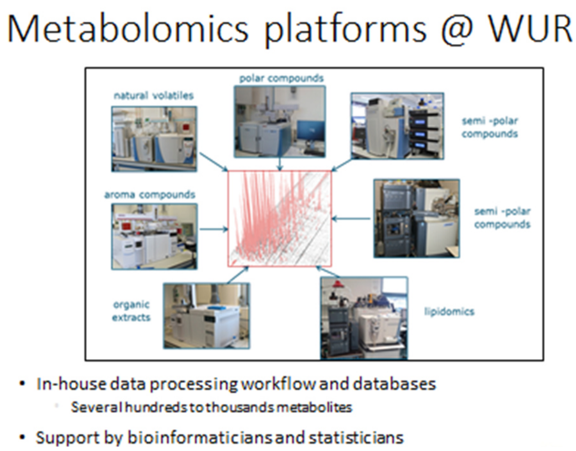

Figure 2. Metabolomics platforms present at Wageningen UR used for the biochemical analysis of duckweed samples.

Digestibility analysis

In the Netherlands Lemna was cultivated on nutrient solution and on bioslurry solution (separated thin fraction of digestate). The nutritional values are determined for Lemna from both cultivations, which makes comparison of the two growing media possible. The cultivated Lemna was dried in an oven, and dry matter content was determined. Dried samples were analysed on three different ways for digestibility:

- Short Boison method: *In vitro* simulations of stomach and small intestine (with phosphate buffer, HCL and Pancreatine solutions) of for instance chicken, pigs and ducks. By filtering and weighing, the amount of bioavailable material was determined.
- Tilley and Terry: *In vitro* simulation of stomach (with rumen fluid and HCL) of for instance cows, sheep and goat. By filtering and weighing, the amount of bioavailable material was determined.
- Weende: Nutritional compounds in Lemna were determined like ash, protein, fibres, starch and sugars

III. RESULTS AND DISCUSSION

1. Analyse potential productivity of several duckweed species under laboratory conditions when grown on a commercial fertilizer

In the young vegetative growth phase (i.e. without severe inter-specific competition) Lemna plants exhibit extremely high growth rates. Relative growth rates (RGR) of over 500 mg g^{-1} day^{-1}, or doubling times of less than 1.5 days have been reported, making Lemna one of the fastest growing plants on earth (Cheng et al. 2002, Ziegler et al. 2015, cf. van der Werf et al. 1998). However, reported RGR rates are difficult to extrapolate to productivity of a farming system, as these reflect plant growth during the exponential growth phase, whereas maximal productivity per unit of surface area occurs in the linear growth phase (Cheng et al. 2002, Brouwer et al. 2017). In this section we first describe the growth profile of eight selected Lemna species/strains to determine the linear growth phase associated with maximal productivity. Based on the obtained results we developed harvest protocols.

Eight different Lemna species and lines were cultivated as described in Methods. All eight species grew linearly between approx. 400-2500 kg dry weight per ha. The slope of day number versus standing crop gives information on daily productivity and varied between 70-112 kg dry weight per ha per day (Fig. 3). No statistical differences were observed between Lemna minor 8744 and Lemna minor 8627, whereas all others were significantly lower than the best performers L. minor 8744/8627.

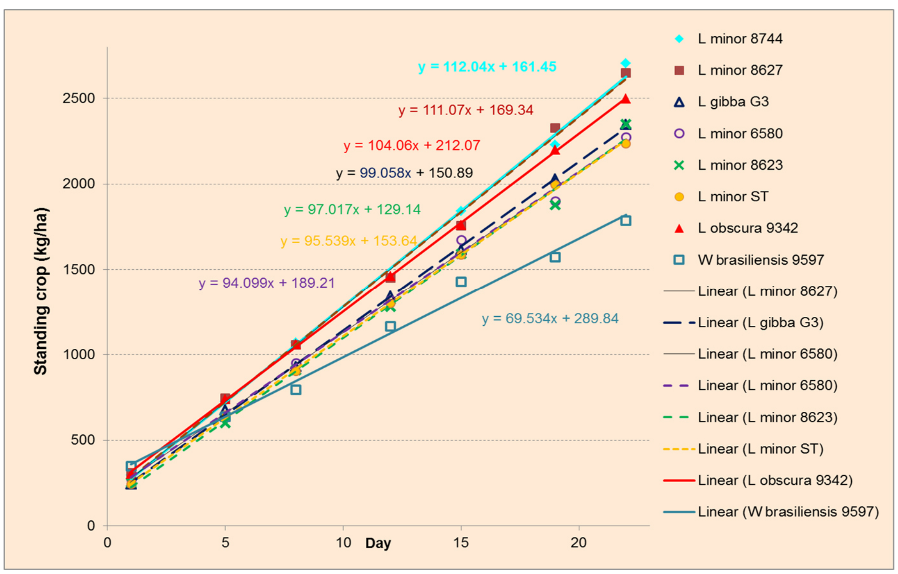

Fig. 3. Standing crop (dry weight) versus time for eight Lemnacaea species. The slope of the linear regression gives the productivity in kg per ha per day.

The first preliminary experiments in Q2 were carried out on a nutrient solution previously used for Azolla species. The newly tested medium resulted in far better performance with respect to daily productivity compared to those grown on the Azolla-medium (Table 2).

Table 2. Daily productivity of several Lemnaceae species grown on a newly tested medium in Q3 and on Azolla-medium in Q2.

Species	Daily productivity, kg ha^{-1} day^{-1}	
	New	Old
Lemna minor 8744	112	
Lemna minor 8627	111	72
Lemna minor 6580	94	
Lemna minor 8623	97	
Lemna minor ST	96	63
Lemna gibba G3	99	79
Lemna obscura 9342	104	77
Wollfia brasiliensis 9597	70	

2. Derive harvesting strategies based on point 1 and selection of most promising species/strain for outdoor experiments

In fig. 4 a theoretical example is given when either 14% of the total surface area is harvested each day or 40% twice a week. In both cases a similar cumulative amount of biomass harvested over time is time achieved, and the amount of biomass shortly before harvest is constant over time, be it that the one for the 14% treatment is significantly lower than the 40% treatment. Several of such harvest schemes were tested for Azolla, both indoors and outdoors and the theory was confirmed (unpublished data and Brouwer et al. 2017).

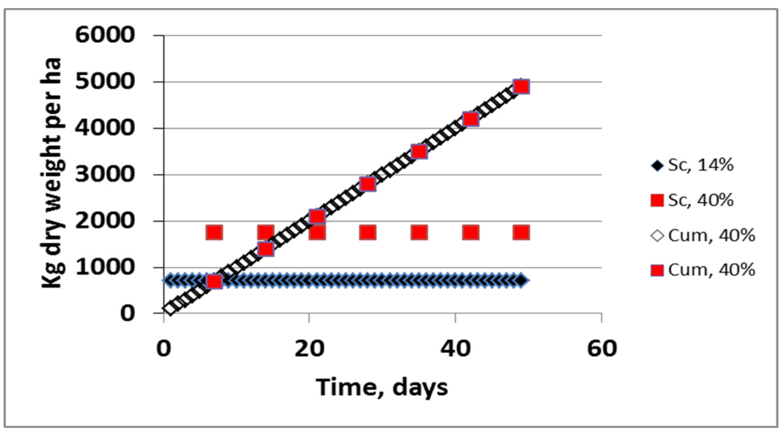

Fig. 4. Standing crop (SC) in time shortly before harvest and cumulative amount of harvested biomass when each day 14% of the surface area was harvested or 40% each week.

For small scale farmers in Indonesia it is highly likely that they want to feed their animals every day with duckweed combined with commercial feed. In the case of 14% harvest of the surface area we would expect a daily harvest of 2.5 and 1.25 kg fresh weight (FW) when growth rates apply of 100 and 50 kg DW/ha/day, respectively for a 15 m2 pond, and 8.3 and 4.2 kg FW for a 50 m2 pond (see section advice on harvesting schemes). Based on growth rate (fig. 3) and protein concentration (part 4) we selected Lemna minor for the outdoor experiment (part 3).

3. Analyse productivity in The Netherlands in 6 m2 outdoor ponds for commercial fertilizer grown and bio-slurry grown duckweed

In the summer of 2016 we started an outdoor experiment in 6 m^2 ponds comparing Lemna grown on nutrient solution versus Lemna grown on bio-slurry. For the commercial fertilizer the same nutrient concentration was used as the one in the laboratory experiments. Based on an initial concentration of nitrogen a starting mixing ratio of 1.4% of the pond volume was chosen, resulting in an ammonium-N concentration of 1.4 mM (and total N of 4 mM which is similar to the total amount of nitrogen in the commercial fertilizer, see table 1). At regular intervals the ammonium concentration was analysed for the bio-slurry treatment and a bio-slurry amount was added such that a target concentration of 1.4 mM ammonium-N would be reached. Similarly, commercial fertilizer concentration was analyzed at the same time, and an amount of nutrients were added, such that the initial concentration would be reached (see table 1). Over the experimental period bio-slurry mixing ratios of 0.3-0.8% were applied. Each week 40% of the surface area was harvested for both treatments. Over time standing crop before and after harvest remained fairly constant (data not shown) and no differences were found in cumulative harvested biomass between the two treatments (fig. 5). In the case standing crop before and after harvest remains fairly constant, the slope of cumulative harvested biomass versus time equals the average growth rate (kg DW/ha/day). During the summer months an average productivity of around 80 kg DW/ha/day was achieved, irrespective of the treatment. These results suggest that bio-slurry, when properly applied, can be used as a valuable nutrient source for Lemna production.

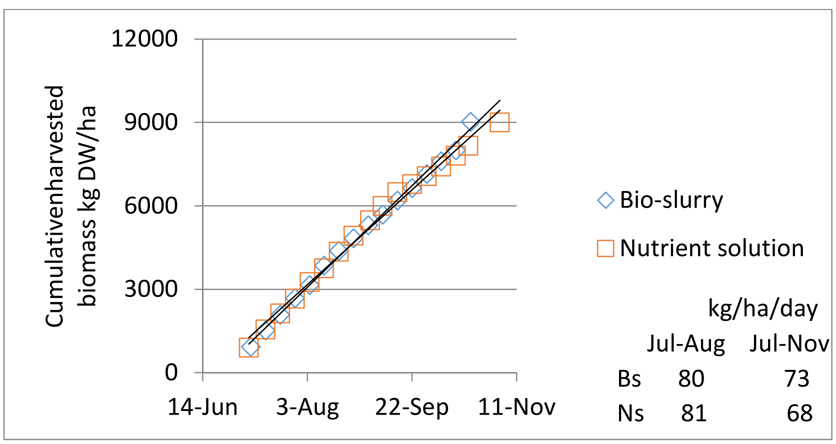

Fig. 5. Cumulative harvested biomass for bio-slurry (Bs) and commercial fertilizer (Ns) grown Lemna.
From the experimental results obtained in The Netherlands advice was given with respect to harvesting and bio-slurry application rate.

4. Analyse nutritional value/ nutrients composition of Lemna minor (grown under point 1)

In 2016 Edelman & Colt published an article (Edelman & Colt, 2016) comparing a duckweed strain with other leafy crops (kale and spinach) and seed crops (wheat, corn, rice, soy and lentils). This publication

shows that leafy crops are more nutritious than seed crops based on the levels of vitamins, minerals and profile of poly unsaturated fatty acids (figure 6). The essential amino acid profiles are also better suited for animal and human consumption than the seed crops. A duckweed species (Wolffia sp) was taken along in their analyses and the results show that duckweed has comparable nutritional qualities as the leaf crop spinach, and is more nutritious than the seed crops, wheat, corn, rice, lentils or soy based on essential amino acid profile, vitamins, minerals and fatty acid profile.
According to this publication, duckweed has a higher level of vitamin A, B1, B2, B5, B6, C, E and K1 compared to wheat, corn, rice, soybean and lentils, and a higher level of the minerals calcium, iron, magnesium, phosphorus, potassium, sodium and zinc compared to all these seed crops. Duckweed has comparable concentrations of these vitamins and minerals to the leavy crops spinach and kale. Especially vitamin B5 and vitamin E are high in duckweed, even higher than in spinach and kale.

TABLE 1 | Nutritional compositions for some seed and leafy plants[a].

	Seed					Leaf		
	Wheat	**Corn**	**Rice**	**Soy**	**Lentil**	**Kale**	**Spinach**	**Duck-weed**
A. VITAMIN CONCENTRATIONS[b]								
Vitamin A, IU (beta-carotene)	9	167	0	114	68	130,000	85,500	77,900
Vitamin B1, mg (thiamine)	0.4	0.2	0.2	0.6	0.6	0.9	0.9	1.1
Vitamin B2, mg (riboflavin)	0.2	0.1	0	1.1	1.3	0.9	1.8	2.8
Vitamin B5, mg (pantothenate)	1	0.5	1.5	1.5	0.4	0.9	0.9	2.1
Vitamin B6, mg (pyridoxal)	0.3	0.3	0.8	0.5	0.5	2.5	1.8	1
Vitamin C, mg (ascorbic acid)	0	0	0	0	2	1014	258	94
Vitamin E, mg (tocopherols)	0.8	0.3	0.2	1.8	–	9.3	18.2	45.7
Vitamin K1, μg (phylloquinone)	1.9	0.2	0	67	–	6900	4400	51
B. MINERAL CONCENTRATIONS[b]								
Calcium, mg	34	6.4	10	195	34	846	1036	607
Iron, mg	3.8	2.2	0.4	6	6.4	8.3	28.4	25.7
Magnesium, mg	120	85	36	407	48	265	827	231
Phosphorus, mg	332	250	100	469	276	519	513	1741
Potassium, mg	405	289	78	2387	664	2769	5840	5319
Sodium, mg	3.1	4.8	0	12.3	5.9	214	827	132
Zinc, mg	3	1.8	0.8	3.7	3.2	3.2	5.5	15
C. OMEGA-6/OMEGA-3 FATTY ACID RATIO								
ω-6/ω-3	19.4	32.2	4.7	7.5	3.7	0.8	0.2	0.3

[a] *Derived from the USDA National Nutrient Database (http://nutritiondata.self.com) for: Wheat flour, whole grain; corn flour, whole grain, yellow; rice flour, white, unenriched; soy flour, full-fat, raw; chick pea, mature seeds, raw; lentil, pink, raw; spinach, raw; broccoli, raw; kale, raw. Data for duckweed determined by Eurofins USA for a local Israeli isolate of dried, raw, Wolffia sp.*
[b] *Values are per 100 g sample. All samples normalized to 10% moisture. "–" indicates a missing or incomplete value.*

Figure 6. Table taken from Edelman & Colt (2016) showing the levels of vitamins, minerals and fatty acid ratio from several seed crops and leaf crops, including the duckweed species Wolffia.

Different components have been analysed in Lemna minor. For nutritional analysis several main food nutrients were analysed such as: protein, starch, dietary fibres, fat and fat composition.
For the different Lemna sp species, which is also used in the pilot experiments in Indonesia, the results can be summarised as follows:

Table 3: Average percentages compounds ***Lemna minor*** strains

Crude protein (Nx6.25)	34-38%
Crude fat	2.1-3.8%
Dietary fibre	26-28%
Starch	1.3-5.3%
Sugars	1.0-2.9%
Carbohydrates (total)	2.1-7.3%

The composition of the crude fat was also analysed of which the results are shown in Figure 7. There is a high percentage of omega-3 fatty acids in relation to omega-6 and omega-9 which is very positive. This leads to good ratio of omega-6/ omega-3 fatty acids of 0.5.

	***L minor* 8627**
Fatty Acid composition	
Omega-3 fatty acids	31.4
Omega-6 fatty acids	17.8
Omega-9 fatty acids	1.9
Ratio Omega-**3**/Omega-**6** fatty acids	1.8
Ratio Omega-**6**/Omega-**3** fatty acids	0.6
Trans fatty acids	0.3
Saturated fatty acids	28.0
Monounsaturated fatty acids	3.1
Polyunsaturated fatty acids	49.3
Unsaturated fatty acids	52.6
Energy value	928
Sodium (Na)	1030

Figure 7. Results of the composition of the crude fat analysed in Lemna minor.

Information gained from farmers with duck farms that join the GADING project revealed that ducks fed on duckweed produce eggs with more orange colored yolk. This can be explained by the fact that duckweed contains a relative high level of carotenoids and vitamin E (as is found in carrots). This is shown in Figure 8 where the results are presented of the LCMS analysis of different carotenoids that are present in duckweed: lutein, beta-carotene, zeaxanthin, alpha-, delta- and gamma-tocopherol (vitamin E). The levels are compared to an other aquatic plant, the water fern Azolla.

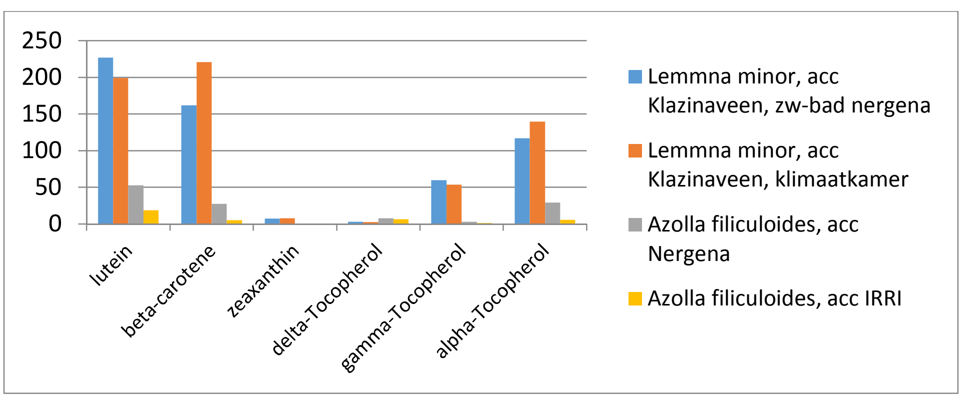

Figure 8. LCMS analysis of carotenoids present in duckweed and Azolla.

An effect of feeding duckweed to chicken/ birds has been reported before in literature (Moreno et al, 2016; Frederikson et al, 2006; http://post.jagran.com/duckweed-a-future-crop-plant-for-india-1396257399; http://www.mekarn.org/Research/thuyctu.htm); see also figure 9.
Carotenoids are used as poultry feed additives in order to achieve the characteristic yellow-orange colour of egg yolks. The effect of colouring eggs of ducks and chicken fed with duckweed can be explained by the high level of carotenoids present in duckweed.

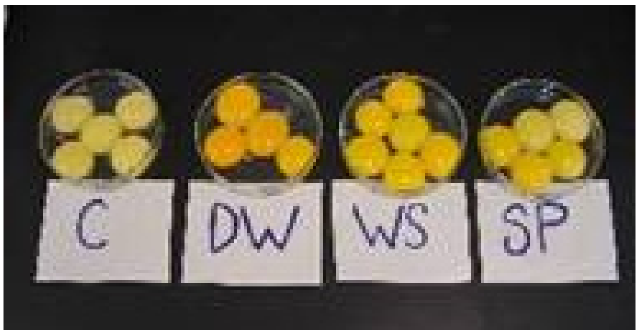

Figure 9. Photo taken from http://www.mekarn.org/Research/thuyctu.htm. The egg yolks from birds fed supplements of duckweed (DW), water spinach WS), sweet potato leaves (SP) or none (C)

Can duckweed be used as feed / partly replacing feed?

Based on literature, the question was answered if duckweed can (partially) replace commercial feed for cows, pigs, ducks, chicken and fish. Protein concentration and the amino acid profile (which are the building blocks of proteins) are an essential quality marker for nutrition. Duckweed contains a high protein level (35-40% under optimal growth conditions. The total amino acid profile is important for a good conversion of feed into animal products. Out of the 20 protein amino acids, 10 are called the essential amino acids because human and animals cannot synthesize them themselves and they have to be taken up via food/ feed. Plants can synthesize all amino acids, but they differ in the overall concentration of all 20 protein amino acids. In some crops one of the essential amino acids is relatively low. The World Health Organisation (WHO) has made a list of the recommended levels of essential amino acids per 100 gr protein (see Table 4). When the levels of essential amino acids in soybean and chickpea flour are compared to data published by Appenroth et al (2017), it is clear that all essential amino acids reach the recommended WHO levels in Duckweed.

Gwaze and Mwale (2015) reviewed the use of Duckweed in pig nutrition and they concluded that duckweed can partially replace commercial feed in piglets, mature pigs and breeding pigs without loss of quantity and or quality. This also holds for fish (e.g. Fasakin et al. 1999, Bairagi et al. 2002, Yilmaz et al. 2004), chickens (e.g. Kusina et al. 1999, Olorunfemi et al. 2006, Chrismadha et al. 2014), ducks (Men et al. 1995, 2001). Appropriate mixing ratios of duckweed and commercial feed per animal type are difficult to derive (and difficult to compare) from the literature as chemical composition and moisture content within and between duckweed species may vary (see refs above). E.g. low protein and high fiber content resulted in decreased animal growth (Haustein et al. 1992). This further highlights the importance of optimal bios-slurry use for duckweed productivity and for its chemical composition. Based on the few publication on this subject (animal trials) it is however concluded by most authors that more research is needed to come up with proper mixing ratios.

Table 4. Comparison of essential amino acid composition of proteins from different sources [g/100 g protein]. Soya and chickpea flour: Commercial flour samples, data from Appenroth et al. (2017). WHO recommendations: WHO (2007). n.d. = not determined

Amino acids	Duckweeds, mean of Appenroth et al. (2017)	Soya flour	Chickpea flour	WHO recommendation
HIS	1.6±0.1	2.7±0.2	2.7±0.1	1.5
ILEU	3.6±0.2	4.2±0.2	4.1±0.2	3.0
LEU	7.4±0.4	7.7±0.4	7.7±0.3	5.9
LYS	4.8±0.8	6.0±0.1	7.0±0.3	4.5
MET + CYS	2.7±0.2	2.9	3.3	2.2
MET	1.7±0.2	1.4±0.1	1.6±0.1	1.6
CYS	1.0±0.1	1.5±0.1	1.7±0.1	0.6
PHE+TYR	7.7±0.6	9.0	8.6	3.8
THR	4.2±0.3	4.1±0.1	3.9±0.2	2.3
VAL	4.6±0.2	4.4±0.4	4.2±0.2	3.9

5. Analyse duckweed biomass grown under several outdoor conditions (grown under point 3).

From the three ponds of duckweed grown on bioslurry (bio-digest) and three ponds of duckweed grown on nutrients, as described under point 3, samples were taken during the growing season, oven-dried and analysed on crude protein content. The results, shown in Table 5, showed that the protein content goes down at the end of the (Dutch) growing season when both light intensity and temperature go down.

Table 5. Crude protein content in bioslurry-grown duckweed and nutrients-grown duckweed during the growing season

Crude protein (Dumas, N x 6.25) in %.						
		Digest			Nutrients	
Harvest date	bath 1	bath 2	bath 3	bath 4	bath 5	bath 6
28/Jul	35.2	34.9	33.4	31.1	29.9	30.7
25/Aug	33.3	33.1	30.4	32.9	33.0	30.7
22/Sep	36.5	36.0	35.6	30.1	30.4	30.0
19 and 20 Oct	29.7	30.8	30.4	26.5	25.2	25.8
Average		33.3			29.7	

Furthermore, crude protein analysis of samples taken from three ponds in which biodigest-grown duckweed and nutrient-grown duckweed was cultivated during the growing season showed that biodigest-grown duckweed showed an overall higher protein content. On average crude protein content of biodigest- grown duckweed was 33.3% versus 29.7% protein in nutrients-grown duckweed. This is a positive result for the pilots in Indonesia where duckweed is grown on bioslurry.

Samples were also analysed for important nutritional factors, such as fat, starch, sugars, fatty acid profile and dietary fibres. Results are presented in Table 6. Overall results are as follows: protein and carbohydrates (starch and sugars) are higher in bio-digest-grown duckweed, whereas fibre content is lower compared to nutrient-grown duckweed. Fat content and the fatty acid profile are comparable.

Table 6 Nutrient content in oven-dried samples from biodigest- and nutrient-grown duckweed during growing season

Big 7 Analysis		28/jul Digest	28/jul Nutrients	25/aug Digest	25/aug Nutrients	22/sep Digest	22/sep Nutrients	19/okt Nutrients
Crude Protein (Dumas, N x 6.25)	%	34.5	30.8	32.1	31.9	35.7	30.1	25.6
Crude Fat	%	2.7	2.5	2.4	2.7	2.5	2.8	2.5
Dietary Fiber	%	34	37	32	36	30	35	34.5
Starch (Enzymatic)	%	1.5	0.4	2.3	< 0.4	2.2	1.7	6.6
Sugars, total as glucose	%	1.3	1.1	2.2	1.2	2	1.1	3.3
Carbohydrates (calculated)	g/100g	2.8	1.5	4.5	1.2	4.2	2.8	9.9
Fatty Acid composition (BF3 method)								
Omega-3 fatty acids	% rel.	35.5	31.8	31.6	31.8	33.6	37.0	36.2
Omega-6 fatty acids	% rel.	18.1	19.2	23.3	19.4	22.1	16.7	20.1
Omega-9 fatty acids	% rel.	2.5	1.8	2.4	1.9	1.8	2.4	3.5
Ratio Omega-3/Omega-6 fatty acids		2.0	1.7	1.4	1.6	1.5	2.2	1.8

This is also a positive result for the Indonesian pilot ponds in which duckweed grows on bioslurry, because it is shown that duckweed grown on bioslurry has a slightly higher crude protein content, and a slightly higher starch and sugar content and is lower in fibre content, which are all positive for animal nutrition and digestion.

That bioslurry is a very good fertiliser, has been reported before (Bioslurry: a supreme fertilizer. A study on bioslurry results and uses. http://www.ourenergypolicy.org/wp-content/uploads/2014/05/bioslurry.pdf). And a positive effect of the use of bioslurry on protein content in duckweed has also been reported before (Lampieu et al, 2004; Chau, 1998).

The samples taken from the ponds with bio slurry and with nutrient solution were oven-dried before analysis. Also the samples from the Indonesian ponds have been oven-dried before sending to the WUR and before analysis. Oven-drying has no effect on protein content, fat composition, starch and sugar content. It also does not affect many health-promoting metabolites. However, some metabolites, such as vitamin C, might be sensitive to drying procedure at 40 degrees C. This is well-known that some metabolites, such as volatile components and metabolites that can be enzymatically converted, will be affected by oven-drying. Still, oven-drying (or sun-drying) is a well-known method to store and preserve plant material. In order to know how large the effect for duckweed might be, fresh samples that were freeze-dried were compared to the same oven-dried samples after the same untargeted metabolomics analysis.
Using untargeted metabolomics the effect of oven-drying versus fresh (freeze-dried) material was analysed for hundreds of different metabolites. A principal component analysis showed that oven drying has an effect on the metabolite profile. However, the different strains that were analysed are in the same way correlated when freeze-dried as when oven-dried. This shows that the way of drying has an effect on some metabolites but overall it does not influence the variation between different *Lemna minor* strains.

Some compounds are effected, but most of them are not. This information is important to know because samples coming from Indonesia have to be oven-dried for transport. Therefore, the analyses performed at WUR on the bioslurry-grown and the nutrients-grown duckweed was also using oven-drying before analysis.

Duckweed is known for its high levels of carotenoids. In this family of compounds, several vitamins are present and other health-promoting or high-value compounds. The concentration of a group of carotenoids was analysed in indoor cultivated duckweed.
Beta-carotene is also known as (pro)vitamin A which has a concentration of 30 mg per 100 gram dry weight. Alpha- and beta-tocopherol is also known as vitamin E which has a combined concentration of about 60 mg per 100 gram dry weight. The total level of carotenoids is high compared to other feed and food crops (20 times compared to soybean and 4 times compared to spinach). Carotenoids give the orange color to carrots and give the color to tomatoes. The high level of carotenoids can explain the orange-colored egg yolk in eggs of animals partly fed on duckweed (already explained under 4.).

Another interesting group of plant compounds with putative health-promoting activities are the group of the flavonoids (also present in red fruit). The flavonoids in duckweed mainly consist of apigenin and luteolin. Analysis of these compounds in the bio-slurry- and nutrient-grown duckweed samples showed that the level of each of them is about 500 mg/ 100 gram DW. The nutrient-grown duckweed seems to have more luteolin. At the end of the growing season in the Netherlands, the levels go down. Luteolin (https://www.sciencedirect.com/topics/neuroscience/luteolin) and apigenin are a specific type of flavonoids that can be found in several vegetables and are associated with decreased risk of cancer, cardiovascular and inflammatory diseases in humans. https://www.ars.usda.gov/ARSUserFiles/80400525/Articles/AICR03_VegFlav.pdf . Miean and Mohamed (2001) analysed 62 edible tropical plants for flavonoid content. Not all plants contain luteolin and apigenin (but other types of flavonoids. The levels in duckweed are 2-10 times higher compared to other vegetable crops. The health-promoting effect of these two compounds for humans will most probably also hold for animals consuming these compounds since many studies analysing their bioactivity have been performed in lab animals (mice, rats).

6. Analyse nutrients composition and heavy metals of duckweed cultivated in Indonesia at different pilot plants

Oven-dried and grinded samples from pilots in Indonesia (Table 7) were received end of March and analysed for nutritional macro-nutrients such as proteins, fat, starch, carbohydrates, dietary fibres (Table 8 and Figure 10). The same samples were analysed on total amino acid profile, on micro-nutrients (minerals) and on heavy metals concentration. The levels of nutritional compounds and minerals/ heavy metals varies between the locations and the concentration of nutritional compounds also varies between the different harvests per location, which could be due to non-standardised growth conditions. The addition of bio-slurry and the level of harvesting needs to be standardised and controlled by measurements of nutrient-content of the pond water.

Table 7. Samples obtained from different locations in Indonesia, harvested at different dates and are sun dried or oven dried.

	Label given in Indonesia	Description	Sampling date	Name given
HIVOS 1	Mauhau-Sumba, 11 November 2016	NTT. Sun dried, Farmer name: John	11 Nov. 2016	Sumba
HIVOS 2	Mauhau-Sumba, 15 December 2016	NTT. Sun dried, farmer name: John	15 Dec. 2016	
HIVOS 3	Mauhau-Sumba, 13 Februari 2017	NTT. Sun dried, Farmer name: John	13 Feb. 2017	
HIVOS 4	Sample I Lombok NTB	Sun dried, Farmer name: H. Badri	7 Feb. 2017	Lombok
HIVOS 5	Sample II Lombok NTB	Sun dried, Farmer name: H. Badri	10 Feb. 2017	
HIVOS 6	Sample III Lombok NTB	Sun dried, Famer name: H. Badri	15 Feb. 2017	
HIVOS 7	Oven sample Batch II, Yogya	Jogjakarta, Oven dried 35°C, Famer name: Suranto	2 Dec. 2016	Jogja, oven
HIVOS 8	Lemna sp. Pengeringan, penyerbukan, kode 17010100126, berat bahan awal 350	Jogjakarta, Oven dried 35°C, Farmer name: Suranto	27 Jan. 2017	

	g, suhu pemanas 35°C Lama pemanasan 65 jam Berat serbuk 21.85 gram 27-1-2017			
HIVOS 9	Lemna sp. Pengeringan, penyerbukan, kode 17020100167, berat bahan awal 350 g, suhu pemanas 35°C Lama pemanasan 65 jam Berat serbuk 21.85 gram 3-2-2017	Jogjakarta, Oven dried 35°C, Farmer name: Suranto	3 Feb. 2017	
HIVOS 10	Sinar Matahari Batch II, Yogya	Jogjakarta, Sun dried, Farmer name: Suranto	2 Dec. 2016	Jogja, sun
HIVOS 11	Sinar Matahari 3 (setelah oven rusak) DIY	Jogjakarta, Sun dried, Farmer name: Suranto	27 Jan. 2017	
HIVOS 12	Sinar Matahari 4 (setelah oven rusak) DIY	Jogjakarta, Sun dried, Farmer name: Suranto	3 Feb. 2017	
HIVOS 13	Batch 1	Lembang, Oven dried 35°C, Farmer name: Cecep Wadayana.	7 Feb. 2017	Name given
HIVOS 14	Batch 2	Lembang, Oven dried 35°C, Farmer name: Cecep Wadayana	20 Feb. 2017	
HIVOS 15	Batch 3	Lembang, Oven dried 35°C, Farmer name: Cecep Wadayana.	27 Feb. 2017	

Crude protein content ranges between 14 and 29.7% in the Indonesian duckweed samples; crude fat between 0.3 and 3.2 %; dietary fibres between 26.5 and 45.2%; starch between 0.2 and 4.3%; carbohydrates between 0 and 4.3%. This is a big range of variation. Comparing these values for the macro-nutrients with the Duckweed grown on bio-slurry in the outdoor pools in the Netherlands shows that the protein content is overall a bit lower in the Indonesia samples, dietary fibres are comparable (but higher when protein content is low), crude fat in several Indonesia samples lower, and carbohydrate levels are very variable in the Indonesia samples and only in one sample higher than the WUR samples.

Table 8. Values for Crude Protein, Crude Fat, Dietary Fibre, Crude Ash, Starch and Carbohydrates of samples from Indonesian pilot ponds.

	Crude Protein	Crude Fat	Dietary Fiber	Crude Ash	Starch	Carbohydrates
	(Dumas, N x 6.25)	(Petroleum etherextraction)	(AOAC 991.43)	(550 °C)	(Enzymatic)	(calculated)
	%	%	%	%	%	g/100g
HIVOS 1	13.2	0.8	44.5	19.5	2.2	2.2
HIVOS 2	26.7	1.5	34.5	26.9	0.9	0.9
HIVOS 3	14.0	1.6	43.0	20.4	4.3	4.3
HIVOS 4	27.7	1.5	45.2	16.0	0.6	0.6
HIVOS 5	29.7	1.3	36.4	15.4	0.7	0.7
HIVOS 6	31.8	2.0	33.2	13.5	1.1	1.1
HIVOS 7	27.7	0.4	30.6	34.5	0.4	0.0
HIVOS 8	28.1	3.2	33.2	16.6	0.2	0.2
HIVOS 9	26.0	2.7	36.2	16.8	0.7	0.7
HIVOS 10	19.2	0.3	26.5	44.5	0.2	0.2
HIVOS 11	25.4	0.8	39.6	25.9	0.4	0.0
HIVOS 12	27.4	0.9	39.1	23.2	0.2	0.2
HIVOS 13	28.5	2.7	32.6	14.0	2.8	2.8
HIVOS 14	25.0	2.7	38.8	15.5	2.1	2.1
HIVOS 15	25.2	2.3	38.2	16.0	1.1	1.1

Values in red are 'lower than' levels.

Based on the percentages of proteins present in these samples, of which most are in the range of 25-30% protein, duckweed grown in the pilot ponds can be very well used as feed. Three samples have a very low protein content (Hivos 1 and 3 from Sumba, and Hivos 10 from Jogja) which points to problems in, or mismanagement of, the cultivation. The nutrition regime (adding bioslurry) might have went wrong. Luckily, the other samples from the same location do show the proper high protein levels, which points to a cultivation system that is not yet standardised at all locations, but also shows that duckweed can be properly cultivated at all locations in Indonesia.

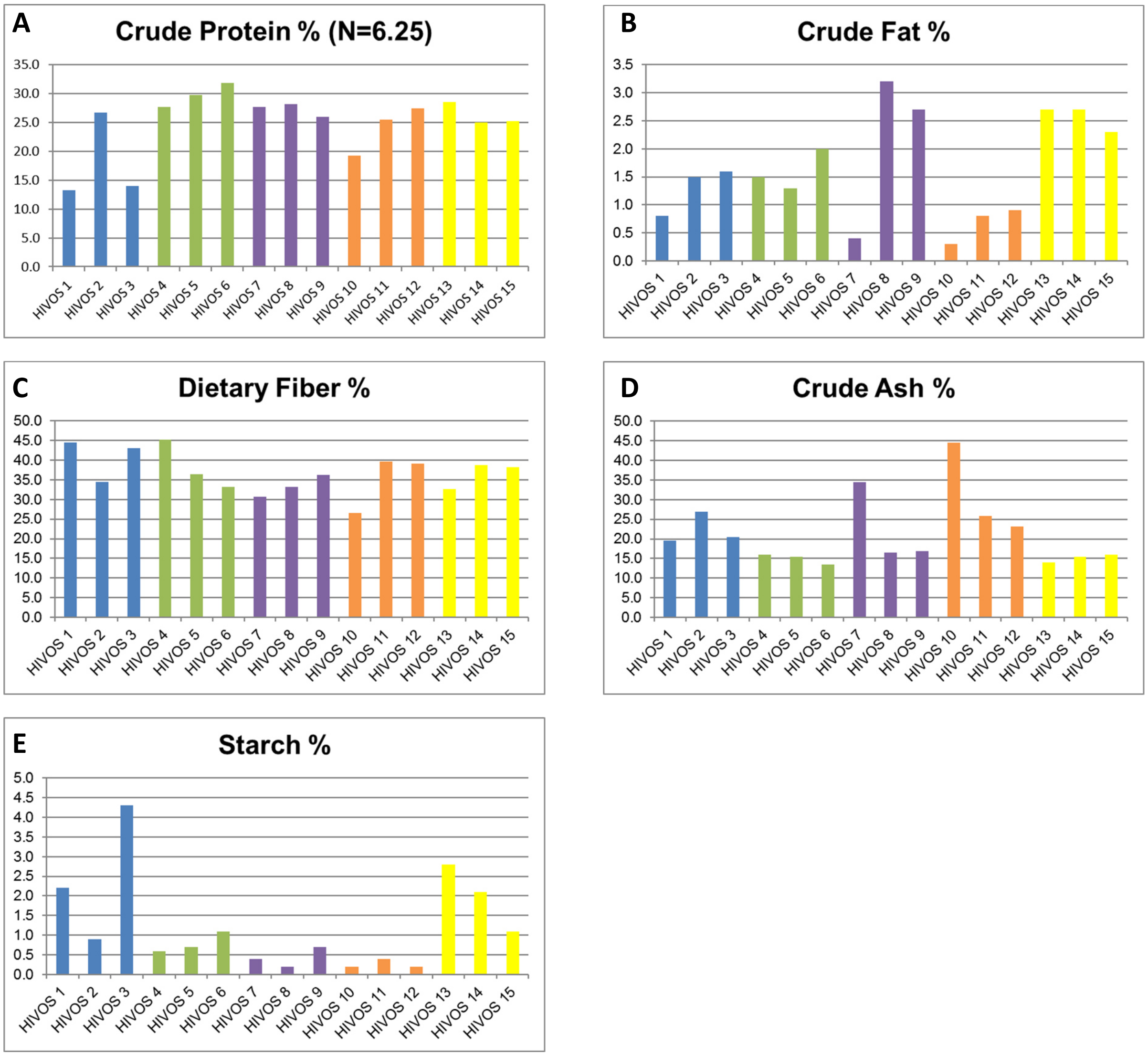

Figure 10. Values for Crude Protein (A), Crude Fat (B), Dietary Fibre (C), Crude Ash (D), and Starch (E). Blue bars: Sumba; green: Lombok; purple: Jogja, oven; orange: Jogja, sun; yellow: Lembang.

Overall, the data show that there is a large variation in concentration of nutritional components between the locations, and between the different harvests per location. This shows that the growing conditions are not standardised yet. Depending on the harvest time per location the levels of the macro-nutrient can vary a lot. This can be clearly seen in the three harvests at Sumba (blue bars) where starch and protein for example vary between the three harvests. But also at all other locations, the harvests differ in macro-nutrient content.

Sun drying versus oven drying (samples 7-9 versus 10-12) does not seem to have a drastic effect on the levels of the macro-nutrients, except for the crude fat content. Values for crude fat seem to be higher in oven dried samples of Jogja compared to sun dried samples (purple versus orange bars).

Comparing the values for the macro-nutrients determined in the samples from the Indonesia plots with the Duckweed grown on bio-slurry in the outdoor pools in the Netherlands (Table 9) shows that the protein content is overall a bit lower in the Indonesia samples, dietary fibres are comparable (but higher when protein content is low), crude fat in several Indonesia samples lower, and carbohydrate levels are very variable in the Indonesia samples and only in one of the three Sumba samples higher than the WUR samples.

Table 9. Data on macro-nutrient content in duckweed grown on bio-slurry versus nutrients, harvested at three time points during growing season in The Netherlands

Big 7 Analysis		28/jul Digest	28/jul Nutrients	25/aug Digest	25/aug Nutrients	22/sep Digest	22/sep Nutrients
Crude Protein (Dumas, N x 6.25)	%	34.5	30.8	32.1	31.9	35.7	30.1
Crude Fat	%	2.7	2.5	2.4	2.7	2.5	2.8
Dietary Fiber	%	34	37	32	36	30	35
Starch (Enzymatic)	%	1.5	0.4	2.3	< 0.4	2.2	1.7
Sugars, total as glucose	%	1.3	1.1	2.2	1.2	2	1.1
Carbohydrates (calculated)	g/100g	2.8	1.5	4.5	1.2	4.2	2.8

The last harvested sample of each series was analysed for total amino acids profile as shown in Table 10.

Table 10. Amino acid concentrations (in g AA/ kg dry weight) in the last harvested sample of each series.

g/kg		HIVOS 3 Sumba	HIVOS 6 Lombok	HIVOS 9 Jogja oven	HIVOS 12 Jogja sun	HIVOS 15 Lembang
Asparaginezuur + Asparagine	Asp+Asn	11.81	26.30	29.00	18.15	27.58
Threonine	Thr	5.51	13.33	10.29	8.55	10.15
Serine	Ser	5.31	13.09	9.76	8.04	9.19
Glutaminezuur + Glutamine	Glu+Gln	14.76	30.34	24.49	18.73	25.81
Proline	Pro	5.75	12.76	10.04	8.50	9.51
Glycine	Gly	6.57	15.46	11.77	10.09	11.81
Alanine	Ala	7.54	17.03	13.05	11.24	13.38
Valine	Val	6.73	16.47	13.39	10.64	13.62
Isoleucine	Ile	5.19	13.06	10.62	8.25	10.99
Leucine	Leu	9.78	24.58	19.10	15.54	18.57
Tyrosine	Tyr	4.08	10.40	7.72	5.84	6.66
Phenylalanine	Phe	5.81	15.23	12.05	9.88	11.88
Lysine	Lys	5.33	16.00	12.54	7.85	13.68
Histidine	His	1.69	4.07	4.13	2.79	4.57
Arginine	Arg	6.02	17.14	13.22	9.37	13.65
Cysteïne	Cys	1.57	3.22	3.08	2.57	2.67
Methionine	Met	2.02	4.61	3.90	2.76	3.61
Tryptofaan	Trp	1.83	4.11	3.75	2.83	3.62

The trend for amino acid profile (differences in levels between the different amino acids) is the same for all analysed samples. There is variation between the samples, but this is correlated with the differences in the protein levels of these samples. Amino acids levels are the lowest in sample 3, which is also the sample with the lowest protein content.

The overall amino acid profile of the Indonesia duckweed samples is comparable to the duckweed samples grown at WUR. Duckweed is a good source of especially the essential amino acids that humans and animals cannot synthesize themselves and have to be taken up from food/ feed.

Three samples of each series were mixed equally and analysed for heavy metals (Arsenic, Cadmium, Mercury, Lead) and minerals (Sodium, Magnesium, Phosphorus, Potassium, Calcium, Manganese, Iron, Copper, Zinc) as shown in Table 11 and figure 11.

Table 11. Concentrations of heavy metals (Arsenic, Cadmium, Mercury, Lead) in mixture of each series.

mg/kg	Arsenic (As)	Cadmium (Cd)	Mercury (Hg)	Lead (Pb)
HIVOS A (HIVOS 1-3)	0.978	<0.15	<0.05	0.75
HIVOS B (HIVOS 4-6)	0.214	0.2	<0.05	0.47
HIVOS C (HIVOS 7-9)	1.4	<0.15	<0.05	1.76
HIVOS D (HIVOS 10-12)	1.64	<0.15	<0.05	1.97
HIVOS E (HIVOS 13-15)	0.265	<0.15	<0.05	1.22

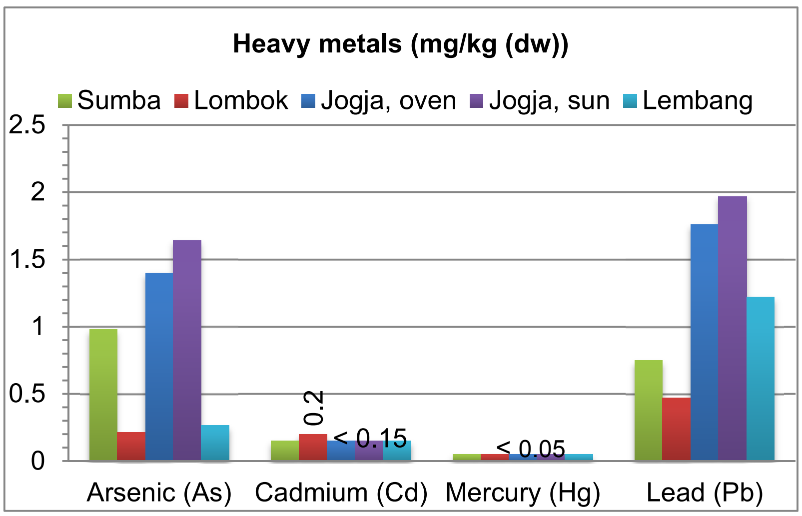

Figure 11. Concentrations of heavy metals (Arsenic, Cadmium, Mercury, Lead) in mixture of each series.

According to European legislation on heavy metals in feed, the maximum content for Arsenic is 4 ppm (4 mg/ kg) in animal feed when the specific feeding stuff is applied as complementary feed with a moisture content of 12%. For Lead this value is 10 ppm, for Mercury this is 0.2 ppm, and for Cadmium this is 0.5 ppm. Values as we have analysed in the Indonesia samples are measures in **dried** duckweed and are **below these maximum tolerable levels**. In the fresh duckweed material the levels are almost even 9 times lower per kg material because of the water content.

What can be noticed is that at location Jogjakarta, farmer Suranto, both the sun dried and oven dried samples contain the highest levels for Arsenic and Lead compared to the other locations, but the levels are still way below maximum tolerable levels. The sample from Lombok, farmer H. Badri, has the lowest levels of these two metals.

The samples were also analysed on mineral content. Results are shown in Table 12 and figure 12.

Table 12. Concentrations of minerals (Sodium (Na), Magnesium (Mg), Phosphorus (P), Potassium (K), Calcium (Ca), Manganese (Mn), Iron (Fe), Copper (Cu), Zinc (Zn) in mg per kg dry weight) in mixture of each series.

mg/kg	Na	Mg	P	K	Ca	Mn	Fe	Cu	Zn
Sumba (HIVOS 1-3)	6000	7100	7500	47000	34000	780	870	15.6	580
Lombok (HIVOS 4-6)	2100	3400	9300	50000	11400	2100	1050	8.2	37
Jogja, oven (HIVOS 7-9)	1910	3500	3600	46000	12000	390	1540	7.3	35
Jogja, sun (HIVOS 10-12)	3200	3300	4300	44000	13000	410	2600	22	16
Lembang (HIVOS 13-15)	1220	3500	10700	43000	10300	5400	2400	11.2	57

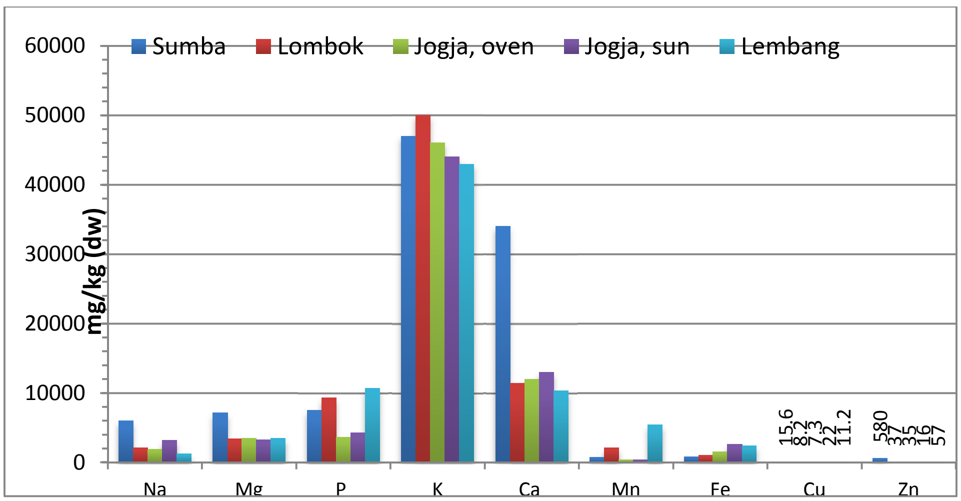

Figure 12. Concentrations of minerals (Sodium (Na), Magnesium (Mg), Phosphorus (P), Potassium (K), Calcium (Ca), Manganese (Mn), Iron (Fe), Copper (Cu), Zinc (Zn)) in pools of each location/ series.

Lembang samples showed a relative high concentration of manganese and Sumba a high concentration of calcium compared to the other locations. This again shows that the cultivation protocol is not standardised, and the regime for addition of bio-slurry is not controlled. Based on simple NH4-analyses of the water in the ponds, proper bioslurry application rates have to be followed as has been advised before and described in this report.

7. Digestibility of Lemna grown under different conditions

Lemna can be used as a feed for cows, chickens, pigs and ducks. Lemna can be cultivated on a small scale at the same location were the animals are kept. The possibilities and value of Lemna as part of the feed composition is not understood well. Goal of this research was to determine the value of Lemna in the feed composition.

Digestibility for chicken, pigs, ducks

Digestibility of the organic matter in the Lemna is shown in Table 13. The percentage of organic matter that is available for the animal is shown. For example, 55% means that the animal will take up 55% of the organic matter. The figures are averages of all results during the growing season 2016 (July untill October). As a reference, grass (of high quality) and soy meal were analysed. The Lemna cultivated on digestate solution is slightly better degradable than cultivation on nutrient solution. Compared to grass a slightly higher digestibility of Lemna is found, when applied as dried feed and when grown under non-limiting conditions.

Table 13. Digestibility of Lemna for chicken, pigs and ducks

	Digestion organic matter %
Lemna nutrient solution	55
Lemna digestate solution	57
Reference: Grass	50
Reference: Soy meal	85

Digestibility for cows, sheep and goats

Digestibility of the organic matter in the Lemna is shown in Table 14. Shown numbers are averages of results during the growing season 2016 (July untill October). The numbers are averages of all results during the growing season 2016 (July untill October). Again, Lemna cultivated on digestate solution is slightly better degradable than after cultivation on nutrient solution. Compared to grass (average derived from literature) slightly lower digestibility of the organic matter in Lemna is found.

Table 14. Digestibility of Lemna organic matter for cows, sheep and goats

	Digestion OM %
Nutrient solution	62
Digestate solution	67
Grass	76#

average figure from literature

Table 15 shows the averages of contents of Lemna for the growing season 2016 (July untill October). The Lemna grown on the digestate solution is higher in protein, starch and sugars. The ash content in the

Lemna grown on the nutrient solution is higher. Compared to measurements for human consumption similar figures were found.

Table 15. Main components in Lemna samples used for digestion analysis

		Lemna digestate	Lemna nutrient
Dry Matter	g/kg	917	917
Protein	g/kg	328	257
Ash	g/kg	152	202
Fibre	g/kg	136	168
Starch	g/kg	53	41
Sugar	g/kg	26	15

As shown already under point 5, Lemna grown on bioslurry contains a higher protein level than Lemna grown on nutrients. Also starch/ sugars are slightly higher, whereas the fibre content is lower in duckweed grown on bioslurry.
The digestibility of Lemna for chicken, pigs and ducks has been tested using *in vitro* digestion methods. Initial findings indicate that Lemna can be valued (based on digestibility and protein content) as a high quality, high protein grass component. For cows, sheep and goats Lemna can be used as a protein component of the feed composition. Feed low in protein, like maize silage, can be used as an energy compound. Lemna cultivated on bioslurry has got better properties considering animal feed than grown on nutrient solution (considering digestibility and components present). This might be due to the lower fibre content. It has been shown before that high fibre content is negatively influencing digestibility of animal feed (Zhang et al, 2013; Owens et al, 2010; Archimède et al, 2011).
The Lemna as animal feed can also be valued for other (not the focus of this research) properties. For instance it has been shown that egg yolk will become more dark yellow/orange when Lemna is fed to chicken and ducks (already discussed at point 4). Also amino acid compositions might be beneficial for the animals.

8. Advice on bio-slurry application rates and harvesting schemes for small scale farmers in Indonesia based on experimental results at WUR and farm visits

When starting a duckweed pond the big question always remains “how much biomass can be harvested?” This will always depend on the productivity of Lemna, on management and environmental conditions. Below a procedure is given that can simply be applied by the farmers

1. Calculations for 10 m^2 pond, with an inoculation of 1 kg FW/m^2, i.e. 10 kg FW per pond at the start
2. Start daily harvest 1 kg FW per 10 m^2 pond (a harvest figure frequently mentioned by farmers). When in time standing crop before and after harvest remains visually constant than we calculate a productivity of 60 kg DW/ha/day, assuming 6% dry weight

3. Now assume a productivity of around 30 kgDW/ha/day is realised at a given farm, i.e. 0.5 kg FW per day per 10 m^2 pond. Calculations show that standing crop after harvest of 1 kg FW per day quickly decreases to 0 after 19 days.
4. Assume a productivity of around 150 kgDW/ha/day, i.e. 2.5 kg FW per day. Now SC after harvest quickly increases by a factor of ~4 after 19 days.
5. So starting with 10 kg FW per pond of 10m2 and a quick decrease of SC after is observed, the advice would be to lower the amount of daily harvested biomass (<1kgFW, e.g. try half)
6. In the case a quick increase is observed, the harvested amount should e.g. doubled.
7. For both points 5&6 keep harvesting until standing crop after harvest remains more less constant. Now the amount harvested reflects the productivity (see text above). For the Dutch situation we observed in our outdoor experiments a productivity of 80 kg DW/ha/day during the summer period when water temperatures varied between 15-25 ^{o}C. Cheng et al. (2002) observed growth rates over more than 200 kg DW/ha/day at average temperatures varying between 25-30 degrees during spring, and regularly exceeded 30 degrees. In fall these rates decreased to 150 kg DW/ha/day due to lower temperatures (20-25 ^{o}C) and light intensities. Chrismadha et al. (2014) and Chrismadha and Mardiati (2012) reported growth rates varying between 28-87 kg DW/ha/day for experiments carried out in Indonesia. These authors indicated that sub-optimal conditions occurred in the Indonesian trials. So when properly managed, productivities in Indonesia should at least equal the ones observed in The Netherlands. In the case calculated productivities are substantially lower than observed in The Netherlands, suboptimal management should be expected. Especially bio-slurry application should than be checked. For this we constructed the so-called ammonium calculator. Based on the information obtained during our joined pilot visits 2016-2017 we concluded that generally ponds lack sufficient nutrients, i.e. pale to yellow fronds and long roots (cf. Van der Werf et al. 1996). Furthermore, based on the amount of biomass harvested in combination with pond size we calculated productivities varying between 16-128 kg DW/ha/day (see table below).

						Bio-slurry application				
						Volume	application	addition per week		productivity
		Area	Village	farm nr		L	per week	% pond volume		kgdw/ha/day
1	21-2-2017	Bandung	Ciater	1		50	7	6.7		93
2	21-2-2017	Bandung	Lembang	1		20	7	3.9		
3	22-2-2017	Garut	"Garut"	1		30	3.5	2.2		128
4	22-2-2017	Garut	"Garut"	2		27	3.5	1.0		120
5	24-2-2017	Yogya	Cangkring	1		10	3.5	0.5		36
6	24-2-2017	Yogya	Cangkring	2		5	2.5	0.2		
7	24-2-2017	Yogya	Balang	1		20	7	3.7		
8	24-2-2017	Yogya	Pagar Jura	1		20	7	3.7		43
9	25-2-2017	Lombok	Pendua	1		15	1	0.3		56
10	25-2-2017	Lombok	Pendua	2		20	1	0.7		92
11	25-2-2017	Lombok	Pendua	3						
12	25-2-2017	Lombok	Pendua	4						
13	27-2-2017	Sumba	near Tambolaka			30	1	0.2		36
14	28-2-2017	Sumba	Werame							72
15	28-2-2017	Sumba	Lewa	1		5	7	0.5		16
16	28-2-2017	Sumba	Lewa	2						
17	28-2-2017	Sumba	Lewa	3						
18	1-3-2017	Sumba	Kamanggih	1						81
19	1-3-2017	Sumba	Kamanggih	2		2	0.25	0.1		122
20	1-3-2017	Sumba	Kataka			25	1	0.9		81
21	1-3-2017	Sumba	near Waingapu			10	0.75	0.1		38

How to apply sufficient bio-slurry?

1. Let us assume that a productivity of around 100 kg DW / ha / day should easily be achieved in Indonesia as found for indoor experiments in The Netherlands. This from here on will be called the "target productivity". The protein concentration of the Lemna biomass varies between 25-40%. This corresponds to a nitrogen concentration ~ 4-6 % of the dry biomass. A concentration of 5% nitrogen (N) is assumed as "target N-concentration".
2. Point 1 result in an uptake of 5 kg N / ha / day. Pond size of the pilots generally varied roughly between 20-40 m^2. As an example we take the 20 m^2 pond. In this case a productivity of 20/10.000 * 100 = 0.2 kg per day per 20 m^2 pond is realized (this is our "target productivity"). This equals 200 g dry weight production per pond per day. Assuming 6% dry weight this equals 3.3 kg fresh weight that can be harvested in a 20 m^2 pond.
3. To sustain the target productivity, a target nitrogen concentration of 5% should be achieved. This would lead to an uptake of 10 g N per 20 m^2 pond per day.
4. Over a one week period 1400 g of dry biomass will be produced and for that target productivity, 70 g of nitrogen would be required to sustain target productivity.
5. To avoid nitrogen depletion after one week of growth at least 100 g of N should be present after addition of bio-slurry. As the concentration varies with volume when a total of 100 g is needed, the target NH4 concentration will decrease with increasing volume, and so with increasing water depth (see table below)

							Target		Target	
Surface ar	depth	Volume	Productivity		N-req		Slurry N		NH4-conc pond	
m2	cm	L	kg/surface area pond		g/pond/week		g/pond/week		mg/L	
20	30	6000	0.2		70		100		21	
20	40	8000	0.2		70		100		16	
20	50	10000	0.2		70		100		13	
20	60	12000	0.2		70		100		11	
20	70	14000	0.2		70		100		9	
20	80	16000	0.2		70		100		8	

6. How much bio-slurry should be applied to reach the target NH4 concentration (further referred to as [NH4]) in the pond when bio-slurry is applied for the first time in a pond? a) analyze [NH4] before application, b) add 1.4% bio-slurry of the pond volume (as was done in The Netherlands), c) analyze [NH4] after application. Now let us assume a pond with a water table of 40 cm (=8000 L) and start [NH4] of 5 mg/L, and after addition of 1.4% bio-slurry (=112 L) a [NH4] of 15 mg/L is measured. So 112 L corresponds with an increase of 10 mg NH4/L. As after addition a [NH4] was measured of 15 mg/L, we are 1 mg/L short compared to target [NH4] of 16 mg/L. So another 1/10*112 L = ~ 11 L should be added to reach the target [NH4].
7. Analyze each week [NH4] and add bio-slurry accordingly, i.e. knowing that 112 L corresponds with an increase of 10 mg NH4/L.
8. Note that this a specific example and that step 6 should be determined for each farm/pond separately.
9. Note that when e.g. surface area doubles productivity per pond doubles and so does N-requirement. Also Target Slurry N has to doubled (see table above), and so Target [NH4] does not change with different surface areas.

10. The water depth of pilot ponds varied between roughly 20 cm and 60 cm, which corresponds to a target NH4 concentration of 24 and 8 mg NH4 / L, respectively. Our present ammonium kit gives the following ranges (mg/L): 0-0.5, 0.5-1, 1-2, 2-5, 5-10. For a given data range it is suggested to use the average. When the measured concentration is higher than 10 mg NH4/L one could also dilute the original sample.

9. Economic analyses of integrated duckweed – animal small scale farming systems

In the Q6 report an economic questionnaire was presented which was recently updated with commercial feed daily intake per animal type.

To analyse the economics of duckweed farming, the amount of protein in the harvested duckweed biomass was compared to the amount of daily protein intake via commercial feed. To analyse how much farmers can save on commercial feed purchase, the following questions were addressed to several farmers in the Java, Sumba, NTB and DIY region:

1. How much fresh weight of Duckweed is harvested and given to the animals and to which type of animals?
2. Daily commercial feed intake by the animals?
3. Protein content of the commercial feed given to the animals?
4. Regional price of the commercial feed?

Based on these questions, the amount of feed protein that can be substituted by duckweed protein can be calculated, and so how much money can be saved by replacing part of the commercial feed by duckweed.

To check whether appropriate growth of duckweed was achieved by the farmers additional questions on pond characteristics (size and volume) and on bio-slurry application rates were addressed. The full questionnaire and calculations derived from it are given tables 1-4. No data were available on protein content of the duckweed. Therefore we assumed 30% protein in the duckweed biomass, which is an average for well-nourished duckweed.

In the Java region 3 farmers were questioned by the regional organic farming officer. All four solely fed duckweed to fish (table 1). Productivity was around 90 kg DW/ha/day, and the amount of bio-slurry applied each 2 weeks varied between 1.3 and 2.6 % of the total pond volume. These data are comparable to the ones found in our experiments in the Netherlands. The daily amount of duckweed given to animals varied between 3-5 kg of fresh weight per day and thus could almost fully replace the commercial feed, leading to an annual saving of roughly 1-1.5 million IDR per year.

In the Sumba region 5 farmers were questioned by the regional organic farming officer. All five gave duckweed to their pigs and one farmer additional also fed duckweed to fish and ducks (table 2). Productivity varied between 75-250 kg DW/ha/day, and the amount of bio-slurry applied every 2 weeks varied between 0.7-2.8% of the total pond volume. The daily amount of duckweed given to animals varied between 1.2-10 kg of fresh weight per day, leading to an annual saving of roughly 0.25-2.5 million

IDR per year. As productivity seems to be optimal, the a return is mainly due to the small surface area (and so the amount of biomass that can be continuously harvested daily) of the ponds used in this region.

In the DYI region also 5 farmers were questioned by the regional organic farming officer. On three farms duckweed was fed to ducks, on one farm to chickens in on the other one to cows (table 3). Except for the farmer that fed duckweed to its cows, none of the farmers purchased commercial feed. Productivity varied between only 30-56 kg DW/ha/day, and the amount of bio-slurry applied every two weeks varied between 2.5-5.1% of the total pond volume. The daily amount of duckweed given to the cows was 1.4 kg of fresh weight per day, leading to an annual saving of roughly 0.2 million IDR per year. This low saving is partly due to the very low duckweed productivity in the pond.

In the NTB region 4 farmers were questioned by the regional organic farming officer. In all cases duckweed was fed solely to fish. Productivity in this region extremely low and varied between only 10-30 kg DW/ha/day, and the amount of bio-slurry applied every two weeks varied between 0.6-0.8% of the total pond volume. The daily amount of duckweed given varied between 0.5-2 kg of fresh weight, leading to an annual saving of roughly 0.08-0.34 million IDR per year. Despite the relatively large surface areas of the ponds, this low saving is mainly to the very low duckweed productivity in the pond.

Conclusion: the low savings observed at several farms is mainly due to the small surface areas of the ponds and/or the low productivity, and so the small amount of biomass that can be harvested daily from the ponds.

In the tables below data in black are the ones provided by the farmers, in red are the calculations based farmer's information.

Table 17

Java

	Unit	Ayin	Dede S	Itang
Pond size	m2	27	35	20
Depth	m	0.35	0.4	0.3
bio-slurry	l/day	12	12.5	11
bio-slurry	%/2weeks	1.8	1.3	2.6
Lemna biomass harvested	kgFW/day	4	5.25	3
Protein content Lemna biomass	%	30	30	30
Daily intake commercial feed				
Ducks	kg			
Chicken	kg			
Cows	kg			
Pigs	kg			
fish	kg	0.5	0.5	0.5
Protein content commercial feed				
Ducks	%			
Chicken	%			

	Cows	%			
	Pigs	%			
	fish	%	15	17	15
Price commercial feed					
	Ducks	IDR/kg			
	Chicken	IDR/kg			
	Cows	IDR/kg			
	Pigs	IDR/kg			
	fish	IDR/kg	7500	7500	7500
Number of					
	Ducks				
	Chicken				
	Cows				
	Pigs				
	fish		500	500	500
Feed purchase		IDR/month	112500	112500	112500
Productivity Lemna biomass		kg DW/ha/day	88.9	90.0	90.0
Lemna biomass harvested		g DW/pond/day	240	315	180
Protein harvested		g /pond/day	72	94.5	54
Commercial feed protein consumed					
	Ducks	g/day			
	Chicken	g/day			
	Cows	g/day			
	Pigs	g/day			
	fish	g/day	75	85	75
Protein replacement					
	Ducks	g/day			
	Chicken	g/day			
	Cows	g/day			
	Pigs	g/day			
	fish	g/day	72	95	54
Feed replacement					
	Ducks	kg/day			
	Chicken	kg/day			
	Cows	kg/day			
	Pigs	kg/day			
	fish	kg/day	0.48	0.56	0.36
IDR saved					
	Ducks	IDR/day	0	0	0
	Chicken	IDR/day	0	0	0
	Cows	IDR/day	0	0	0
	Pigs	IDR/day	0	0	0
	fish	IDR/day	3600	4169	2700

Total	**IDR/day**	**3600**	**4169**	**2700**
	IDR/month	**109500**	**126811**	**82125**
	IDR/year	**1314000**	**1521728**	**985500**
Feed costs	IDR/year	**1368750**	**1368750**	**1368750**

Table 18

Sumba

Item		Unit					
Farmer			J Lukas	KK Naha	UDMKubu	UTunggu	KL Amah
Pond size		m2	24	13	12	9	11
depth		m	0.25	0.38	0.27	0.32	0.35
bio-slurry		l/day	8.4	10	5.7	1.4	5.7
bio-slurry		%/2weeks	2.0	2.8	2.5	0.7	2.1
Lemna biomass harvested		kgFW/day	10	3	1.5	1.25	2
Protein content Lemna biomass		%	30	30	30	30	30
Daily intake commercial feed							
	Ducks	kg	0.5				
	Chicken	kg					
	Cows	kg					
	Pigs	kg	1	8	10	5	6
	fish	kg	1.5				
Protein content commercial feed							
	Ducks	%	16.1				
	Chicken	%					
	Cows	%					
	Pigs	%	16.1	16.1	16.1	14.5	16.1
	fish	%	27				
Price commercial feed							
	Ducks	IDR/kg	3000				
	Chicken	IDR/kg					
	Cows	IDR/kg					
	Pigs	IDR/kg	4000	4000	4000	5000	4000
	fish	IDR/kg	12500				
Number of							
	Ducks		7				
	Chicken						
	Cows						
	Pigs		4	4	9	4	3

	fish		4000	200			
Feed purchase		IDR/month					
Productivity Lemna biomass		kg DW/ha/day	250	138	75	83	109
Lemna biomass harvested		g DW/pond/day	600	180	90	75	120
Protein harvested		g /pond/day	180	54	27	22.5	36
Commercial feed protein consumed							
	Ducks	g/day	80.5				
	Chicken	g/day					
	Cows	g/day					
	Pigs	g/day	161	1288	1610	725	966
	fish		405				
Protein replacement							
	Ducks	g/day	22				
	Chicken	g/day					
	Cows	g/day					
	Pigs	g/day	45	54	27	23	36
	fish	g/day	113				
Feed replacement							
	Ducks	kg/day	0.139				
	Chicken	kg/day					
	Cows	kg/day					
	Pigs	kg/day	0.28	0.34	0.17	0.16	0.22
	fish	kg/day	0.42				
IDR saved							
	Ducks	IDR/day	418				
	Chicken	IDR/day	0				
	Cows	IDR/day	0				
	Pigs	IDR/day	1114	1342	671	776	894
	fish	IDR/day	5220				
	Total	**IDR/day**	**6752**	**1342**	**671**	**776**	**894**
		IDR/month	**205365**	**40807**	**20404**	**23599**	**27205**
		IDR/year	**2464385**	**489689**	**244845**	**283190**	**326460**
Feed costs		IDR/year	**2007500**	**11680000**	**14600000**	**9125000**	**8760000**

Table 19

DYI

Item	Unit					
Farmer		Sayemi	Suparmi	R Kristiningsih	W Lesltari	S Ngatiyah
Pond size	m2	15	18	15	24	15
depth	m	0.4	0.4	0.4	0.4	0.4
bio-slurry	l/day	21	26	21	17	21
bio-slurry	%/2weeks	4.9	5.1	4.9	2.5	4.9
Lemna biomass harvested	kgFW/day	0.75	0.9	1.4	1.2	0.75
Protein content Lemna biomass	%	30	30	30	30	30
Daily intake commercial feed						
Ducks	kg					
Chicken	kg					
Cows	kg				5	
Pigs	kg					
fish	kg					
Protein content commercial feed						
Ducks	%					
Chicken	%					
Cows	%				16	
Pigs	%					
fish	%					
Price commercial feed						
Ducks	IDR/kg					
Chicken	IDR/kg					
Cows	IDR/kg				4000	
Pigs	IDR/kg					
fish	IDR/kg					
Number of						
Ducks		10				
Chicken		0				
Cows		2				
Pigs		0				
fish		0				
Feed purchase	IDR/month					
Productivity Lemna biomass	kg DW/ha/day	30	30	56	30	30
Lemna biomass harvested	g DW/pond/day	45	54	84	72	45
Protein harvested	g /pond/day	13.5	16.2	25.2	21.6	13.5
Commercial feed protein consumed						
Ducks	g/day					
Chicken	g/day					

	Cows	g/day				800	
	Pigs	g/day					
	fish						
Protein replacement							
	Ducks	g/day					
	Chicken	g/day					
	Cows	g/day				22	
	Pigs	g/day					
	fish						
Feed replacement							
	Ducks	kg/day					
	Chicken	kg/day					
	Cows	kg/day				0.14	
	Pigs	kg/day					
	fish	kg/day					
IDR saved							
	Ducks	IDR/day					
	Chicken	IDR/day					
	Cows	IDR/day				540	
	Pigs	IDR/day					
	fish						
	Total	**IDR/day**				**540**	
		IDR/month				**16425**	
		IDR/year				**197100**	
Feed costs		IDR/year	**0**	**0**	**0**	**7300000**	**0**

Table 20

NTB

Item	Unit				
Pond					
size	m2	21	40	60	12
depth	m	0.6	0.6	0.6	0.5
bio-slurry	l/day	5	10		3.5
bio-slurry	%/2weeks	0.6	0.6		0.8
Lemna biomass harvested	kgFW/day	1	2	1	0.5
Protein content Lemna biomass	%	30	30	30	30
Daily intake commercial feed					
Ducks	kg				
Chicken	kg				
Cows	kg				
Pigs	kg				
fish	kg	4	5	5	1
Protein content commercial feed					
Ducks	%				
Chicken	%				
Cows	%				
Pigs	%				
fish	%	35	35	35	35
Price commercial feed					
Ducks	IDR/kg				
Chicken	IDR/kg				
Cows	IDR/kg				
Pigs	IDR/kg				
fish	IDR/kg	8933	9000	9000	9000
Number of					
Ducks					
Chicken					
Cows					
Pigs					
fish		8000	5000	5000	2000
Feed purchase	IDR/month	1071960	1350000		1080000
Productivity Lemna biomass	kg DW/ha/day	29	30	10	25
Lemna biomass harvested	g DW/pond/day	60	120	60	30

Protein harvested	g /pond/day	18	36	18	9
Commercial feed protein consumed					
Ducks	g/day				
Chicken	g/day				
Cows	g/day				
Pigs	g/day				
fish		1400	1750	1750	350
Protein replacement					
Ducks	g/day				
Chicken	g/day				
Cows	g/day				
Pigs	g/day				
fish	g/day	18	36	18	9
Feed replacement					
Ducks	kg/day				
Chicken	kg/day				
Cows	kg/day				
Pigs	kg/day				
fish	kg/day	0.05	0.10	0.05	0.03
IDR saved					
Ducks	IDR/day	0	0	0	0
Chicken	IDR/day	0	0	0	0
Cows	IDR/day	0	0	0	0
Pigs	IDR/day	0	0	0	0
fish		459	926	463	231
Total	**IDR/day**	**459**	**926**	**463**	**231**
	IDR/month	**13974**	**28157**	**14079**	**7039**
	IDR/year	**167685**	**337886**	**168943**	**84471**
		1304218	**1642500**	**1642500**	**328500**
Feed costs	IDR/year	**0**	**0**	**0**	**0**

IV. OVERALL CONCLUSION

Nutritional analyses, both on fresh material and on dried material, have been performed on Lemna minor strains grown in growth chambers. On average, protein content ranges from 34-39%. Next to proteins, also other nutritional components are present, such as fat (3-4%), dietary fibres (25%), starch (1.5-5.5%) and carbohydrates (2-7%). The amino acid levels are comparable to soybean, and the fatty acid composition has a very good ratio of omega-6:omega-3. Duckweed contains relatively high levels of carotenoids and vitamin E, which can result in high quality duck eggs with orange yolk when ducks are fed on duckweed. Duckweed also contains vitamin A,B1, B2, B5, B6, C, and K1 plus polyphenolic compounds that have anti-oxidant activity. Based on indoor growth analyses, harvest schemes were derived for outdoor experiments.

From the end of July until beginning of November each month biomass was collected from outdoors cultivation in the Netherlands for chemical analyses in order to analyse effect of seasonal variation on chemical composition, and to analyse the effect of adding nutrient solution versus bioslurry to the water on the chemical composition of the duckweed grown on it. No differences in productivity were observed between bio-slurry and commercial fertilizer grown duckweeds, suggesting that bio-slurry can perfectly well be used as nutrient source for duckweed production.

Crude protein content of these samples varied between 30 and 37% in the bioslurry-grown duckweed, and between 25 and 33% in the nutrient-grown duckweed. It turns out that growth on bioslurry is better for the protein content of duckweed, Lemna minor. Crude fat and carbohydrates content is also slightly higher in bioslurry-grown duckweed, whereas the level of dietary fibres is lower in bioslurry-grown duckweed. Amino acids profile between bioslurry- and nutrients-grown duckweed is not different. The profile is still comparable, only the total level of crude protein differs between bioslurry-grown duckweed (higher content) and nutrient-grown duckweed. Macro- and micro-elements, including heavy metals were analysed for the bioslurry added to the ponds in the Netherlands. Based on the outcome of all our analyses we concluded that bio-slurry can safely be used as a nutrient source to maximally grow Lemna under the prevailing environmental conditions. Furthermore, the bioslurry-grown duckweed showed to have a higher protein content, a (slightly) higher carbohydrate/ starch content and a lower fibre content.

The digestibility of Lemna for chicken, pigs and ducks has been tested using *in vitro* digestion methods. Initial findings indicate that Lemna can be valued (based on digestibility and protein content) as a high quality, high protein grass component. For cows, sheep and goats Lemna can be used as a protein component of the feed composition. Feed low in protein, like maize silage, can be used as an energy compound. Lemna cultivated on bioslurry has got better properties considering animal feed than grown on nutrient solution (considering digestibility and components present). This might be due to the lower fibre content. It has been shown before that high fibre content is negatively influencing digestibility of animal feed. Lemna as animal feed can also be valued for other properties. For instance it has been shown that egg yolk will become more dark yellow/orange when Lemna is fed to chicken and ducks.

Dried samples from Indonesian pilot ponds at four locations were sent to the Netherlands and subjected to nutritional and biochemical analysis. The levels of nutritional compounds and minerals/ heavy metals showed to vary between the locations and the concentration of nutritional compounds also varies between the different harvests per location, which could be due to non-standardised growth conditions. Crude protein content ranged between 14 and 29.7% in the analysed duckweed samples; crude fat between 0.3 and 3.2 %; dietary fibres between 26.5 and 45.2%; starch between 0.2 and 4.3%; carbohydrates between 0 and 4.3%. This is a huge range of variation. Comparing these values for the macro-nutrients with the Duckweed grown on bio-slurry in the outdoor pools in the Netherlands shows that the protein content is overall a bit lower in the Indonesia samples, dietary fibres are comparable (but higher when protein content is low), crude fat in several Indonesia samples are lower, and carbohydrate levels are very variable in the Indonesia samples and only in one sample higher than the WUR samples. Concentration of micro-nutrients (minerals) also varies between the different locations. Results show that duckweed is a good source of minerals. Calcium level is almost three times higher in the Sumba samples which points to a high concentration of calcium in the pond water. Heavy metals content is also variable between the locations, but in all cases low and way below the maximum acceptable levels for animal feed according to European legislation.

Based on these outdoor experiments advice was given for proper bio-slurry application rates and simple rules for how much biomass can be harvested. Based on the visits 2016-2017 it is concluded that until October 2017 management of ponds was such that proper productivities have not been reached yet. It is advised that the harvest procedure and the use of the NH4-calculator is followed according the description in this document. Based on the data obtained under above mentioned conditions, the final economic analysis will be presented soon.

The economic analyses show that the low savings observed at several farms is mainly due to the small surface areas of the ponds and/or the low productivity, and so the small amount of biomass that can be harvested daily from the ponds.

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