Opportunities for micro algae as ingredient in animal diets

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1 Introduction

1.1 Occasion

Within the Implementation Agenda Sustainable Livestock Farming (Uitvoeringsagenda Duurzame Veehouderij; UDV) important targets are the closure of the mineral cycles and the sustainability of animal feed. Topics in this respect are: sustainable resources for livestock feed, optimization of loop systems/recycling of mineral cycles, import of feed, export of manure, application of manure for plant production and research on protein sources through the bio-based economy. Within the context of the UDV, Nevedi (branch organisation of the animal feed industry) and Natuur & Milieu (NGO for Nature & Environment) have started a project focused on the sustainability of feed for livestock. At a strategic level, a search was started for feed alternatives of European origin as replacement for imported soy products. Within this project there is, among other, a need for an assessment of the opportunities for the use of algae in diets for livestock. Algae can become an important high-protein source in the composition of diets for farm animals. Algae are able to use watery nutrient flows very well. A pilot study already has already been started, in which algae are produced in a closed nutrient cycle system for application in the diet of veal calves. This system makes use of energy (heat), which comes available by fermentation of the urine of the calves and digestate. In speciality feeds (for example horse diets produced by Van Benthem) algae are applied because of the health promoting properties (vitamins, etc.) of the algae. Until now, algae are not applied in the diets as substitution for conventional feed proteins. Currently this is not cost-effective. Studies show that especially the costs for production of algae still hampers a further development of algae production. Production costs often are far in excess of the current prices for the conventional protein sources. And it will not be easy to reduce these costs in short term.

1.2 Aim

The aim of this project is to identify the opportunities for application of algae in feed. The benefits and disadvantages of micro algae as nutrient source in livestock diets will be described, as well as the operational aspects related to application in farm animal diets. These benefits and disadvantages will be summarised in a leaflet. This report provides all background information.

1.3 Structure of the report

The current production of algae and the application in animal feed, both globally and in The Netherlands, are reviewed in Chapter 2. The involved legislative aspects are discussed in Chapter 3. The nutritional value and the health stimulating properties are described in Chapter 4 and 5. Subsequently, the research results per group of animal species are discussed (Chapter 6). In the following two chapters (7 and 8) the economic and sustainable aspects are described. Finally, the opportunities and challenges for the application of algae in feed are indicated (Chapter 9 and 10).

2 Current production figures and applications of algae in animal feed

2.1 Global production and applications of algae

Already for centuries, micro algae have been used by indigenous populations, whereas the cultivation of micro algae is only a few decades old. Of the 30.000 species, that are presumed to exist, only a few thousand are conserved in collections, a few hundred are examined on chemical content, and just a few are grown on an industrial scale. The most relevant micro algae from a biotechnological point of view are the green algae (*Chlorophycea*) *Chlorella vulgaris, Haematococcus pluvialis, Dunaliella salina* and the Cyanobacteria *Spirulina maxima*. Especially *Spirulina* is being traded and used on a large scale, particularly as nutrient supplements for humans and as additives in animal feed. Various micro algae species (e.g. *Chlorella, Tetraselmis, Spirulina, Nannochloropsis, Nitzchia, Navicula, Chaetoceros, Scenedesmus, Haematococcus, Crypthecodinium*) and macro algae (for example *Laminaria, Gracilaria, Ulva, Padina, Pavonica*) can be used for in feed application (Gouveia et al., 2008).

In Table 1, the extent and countries of production, and applications of the main algae species are represented. *Spirulina* (*Arthrospira*) is the algae species, which is most produced, followed by *Chlorella (see* table 1).

Table 1: Annual production, country of production, and applications and products of algae per algae species (Kovač et al., 2013).

Algae-species	**Annual production (tons/year)**	**Country of production**	**Applications and products**
Spirulina (Arthrospira)	3.000	China, India, USA, Myanmar, Japan	Human and animal nutrition, cosmetic products (phycobiliproteins, powders, extracts, tablets, drinks, chips, pasta, liquid extracts)
Chlorella sp.	2.000	Taiwan, Germany, Japan	Human nutrition, aquaculture, cosmetic products (tablets, powders, nectar, noodles)
Dunaliella salina	1.200	Australia, Israel, USA, China	Human nutrition, Cosmetic products (ß-carotene, powders)
Aphanizomenon flos-aquae	500	USA	Human nutrition (capsules, crystals, powder)
Haematococcus pluvialis	300	USA, India, Israel	Aquaculture, astaxanthin
Crypthecodinium cohnii	240 tons of DHA oil	USA	DHA oil
Shizochytrium sp.	10 tons of DHA oil	USA	DHA oil

About 30 percent of the current global biomass production of algae is sold for application in animal feed (Gouveia et al., 2008).

2.2 Production and applications of algae in The Netherlands

The cultivation of algae in The Netherlands yet is still limited and mostly takes place in open basins, but also in tube reactors or in plastic bags, which are exposed to sunlight. In The Netherlands, most of the production occurs in experimental facilities, in which often waste products (e.g. CO_2, heat and effluent) are reused from agriculture, industry, waste water purification systems or the transport sector.

According to model calculations based on algae basins at ACRRES (Photo 1) in Lelystad (The Netherlands) the annual biomass yield of algae is 15 tons of dry matter per hectare. In a system with tubular PBR (photo bioreactor) the production is twice as high (31 tons/ha), whereas in a flat panel PBR the production is more than three times as high (52 tons/ha), compared to an open basin. These differences are a result of the differences in efficiency of photosynthesis (PE) of the daylight (1.5% / 3% / 5%, respectively). These yields in biomass are based on the Dutch climate. For instance, based on model calculations, the yield in Curacao would be more than doubled (Spruijt et al., 2014).

Photo 1: Algae basin at ACRRES-Wageningen UR

An example of an algae production facility, which is operating for many years already, can be found at the organic dairy farm Kelstein in Hallum (The Netherlands; Photo 2). At this site, four photo bioreactors and three basin systems are available for algae cultivation. By-products of the biogas-system, which is also located at the site, are used as input for the system. The algae that are cultivated here are mainly applied in feed. In 2011, the Kelstein farm received the GMP+ certificate, which allows application of the algae in a feed factory (www.kelstein.nl). The algae of the Kelstein farm are processed in algae salt licks, and applied as a feed supplement in horse diets by Van Benthem Animal Feed and Fertilizer (www.vbvoer.nl/supplementen).

Photo 2: Algae-basin at the organic dairy farm Kelstein

Veal calves farmer Kroes in Uddel (The Netherlands) cultivates algae and duckweed, thereby using the liquid fraction of the veal calve manure digestate as a nutrient source. The water fraction of the duckweed cultivation, and eventually the urine of the veal calves are used for algae cultivation. After harvesting, the duckweed is fed to the veal calves. The algae fluid is used to fulfil the moisture requirement of the calves, thereby similarly providing nutrients coming from the algae to the calves.

Photo 3: Algae-tube-reactor in the veal stable at the farm of Kroes

3 Legislative aspects related to the application of algae in feed

The legislation concerning the introduction of animal feed on the market is established in the EC Council Regulation 767/2009. An animal diet or animal feed ingredient has to be registered in the list with permitted products through the 'Feed Materials Register' (www.feedmaterialsregister.eu). If an animal diet does not appear in this register, it has to be announced by a notification (art. 24 (part 6), Council regulation 767/2009/EC). Submission of a new animal feed is also possible through this register.

Trading of animal feed also involves food safety requirements (Council Regulation 767/2009/EC (e.g. Art. 4), the General Foodstuff Council regulation (e.g. Art. 15), and the hygiene regulations in Council Regulation 183/2005/EC.

Admittance of animal feed additives is regulated by Council Regulation 1831/2003. For animal feed additives, there is a register with permitted products as well: the European Union Register of Feed Additives (ec.europa.eu/food/food/animalnutrition/feedadditives/registeradditives_en.htm)

In the past, it was not allowed to sell algae as animal feed additive in case that manure or digestate was used as nourishment for the algae (Van Kasteren, 2011). Since 2013, the GMP+ legislation has been changed, and a new standard concerning food safety is established. The legislation for the cultivation of animal feeds is regulated in https://www.gmpplus.org/bestand/4224/gmp-b6---en-20130301.pdf.ashx.
Algae biomass can only be used as dietary ingredient for animal feed if a risk assessment has been carried out on the production method, and if the sellable products are analysed, to insure that they contain a minimum level of certain substances and that they are not contaminated with pathogens. (Van der Weide et al., 2014).

4 Nutritional value of algae

4.1 General

There is a large variation in nutrient composition between the different algae species. As shown in Table 2, the contents of crude protein, carbohydrate and fat are comparable with, or even higher than some conventional used feed ingredients as soybeans, maize and wheat (Lum et al., 2013).

Table 2: *Nutrient composition of conventional foodstuff and several algae-species (% dry matter) (Lum et al., 2013)*

Source	**crude protein**	**carbohydrates**	**fat**
Soybeans	37	30	20
Maize	10	85	4
Wheat	14	84	2
Anabaena cylindrical	43-56	25-30	4-7
Arthrospira maxima (Spirulina)	60-71	13-16	6-7
Chlorella vulgaris	51-58	12-16	14-22
Spirogyra sp.	6-20	33-64	11-21
Synechococcus sp.	73	15	11

4.2 Proteins

Digestible crude protein

In general, the nutritional quality of drum dried algae is approximately 85% of that of casein. As show in Table 3, the NPU (Net Protein Utilization) values for casein are significantly lower than for the mentioned algae species, which indicate that the algae protein is limited by at least one of the essential amino acids, probably methionine. Nitrogen balance studies with *Spirulina* confirmed that this algae with thin cell membrane does not give severe problems with respect to protein utilisation, even not after simply drying in the sun. To summarize, after exclusion of extreme values, it can be concluded that the nutritional quality of the examined algae after suitable processing is comparable or even better than conventional plant proteins (Becker, 2013).

Algae protein originates defatted algae biomass, and this is a nice alternative for soybean meal. Furthermore, after defatting it still contains a high amounts of omega-3 fatty acids. These fatty acids might contribute to the health status of the animal, end enrich the end product, resulting in a higher added value. (Gatrell et al., 2014)

Table 3: *Comparison of BV (biological value), DC (digestibility coefficient) and NPU (Net Protein Utilization) of different algae species which are processed in different ways (Becker, 2013)*

Algae species	**method of processing**	**BV[1]**	**DC[1]**	**NPU[1]**
Caseïne		87.8	95.1	83.4
Chlorella sp.	air dried	52.9		31.4
Chlorella sp.	drum dried	77.9	89.3	69.6
Chlorella 71105	freeze dried		86.0	
Scenedesmus obliquus	air dried	60.0	51.0	31.0
Scenedesmus obliquus	drum dried	81.3	82.8	67.3
Scenedesmus obliquus	drum dried	76.2	88.6	67.5
Scenedesmus obliquus	drum dried	80.0	81.4	65.8
Scenedesmus obliquus	sun dried	72.1	72.5	52.0
Scenedesmus obliquus	sun dried +cooking	71.9	77.1	55.5
Spirulina sp.	Raw	63.0	76.0	48.0
Spirulina sp.	stewed	51.0	74.0	38.0
Spirulina sp.	sun dried	77.6	83.9	65.0
Spirulina sp.	drum dried	68.0	75.5	52.7
Spirulina sp.+ 0.2% Met	drum dried	82.4	75.7	62.4
Chlorella sp.	protein extract	79.9	83.4	66.2
Chlorella sp.	air dried	52.9	–	31.4
Chlorella sp.	drum dried	71.6	79.9	57.1
Coelastrum proboscidium	drum dried	68.2	77.8	53.1
Coelastrum proboscidium	drum dried	75.3	89.2	67.2
Uronema sp.	drum dried	54.9	81.8	44.9

[1]) The BV is a measure for protein utilisation and is calculated based on the difference between the amount of protein ingested by the animal and excreted through faeces and urine. Besides the involved protein source, the remaining fraction of the test diet consists of protein free or protein low ingredients. This method takes into account the endogenous losses. The DC is calculated in a comparable way as the BV, however without taking into account the excretion of protein through the urine. Consequently, this method overestimates the protein utilisation of the animal. The NPU also is a measure for the protein utilisation. This is calculated by determining the ratio between the amount of protein deposited in the animal body and the amount of consumed protein. The protein deposition is determined by slaughtering the animal after the experimental period and analysing the amount of protein in the carcass.

Amino acid profile

Table 4 provides the amino acid profiles of some algae species as compared to soybeans. Compared to the amino acid profile of soybeans, the amounts of the essential amino acids lysine, cysteine and tryptophan are relatively low, while the amounts of the essential amino acids methionine, threonine and isoleucine are comparable or higher. (Becker, 2013)

Table 4: *Amino acid profile of different algae species compared with soya beans (g/100 g protein), (Becker, 2013)*

Protein source	Ile	Leu	Val	Lys	Phe	Tyr	Met	Cys	Try	Thr	Ala	Arg	Asp	Glu	Gly	His	Pro	Ser
Soybeans	5.3	7.7	5.3	6.4	5	3.7	1.3	1.9	1.4	4	5	7.4	1.3	19	4.5	2.6	5.3	5.8
Chlorella vulgaris	3.2	9.5	7	6.4	5.5	2.8	1.3	–	–	5.3	9.4	6.9	9.3	13.7	6.3	2	5	5.8
	3.5	6.1	5.5	10.2	2.8	2.8	1.4	0.79	2.1	2.8	7.7	3.67	4.21	6.67	3.36	3.3	7.2	3.3
	3.76	7.78	5.27	5.73	7.71		16.02		8.5	5.1	7.5	5.4	8.1	13.7	6.1	1.8	7.7	4.6
Dunaliella bardawil	4.2	11	5.8	7	5.8	3.7	2.3	1.2	0.7	5.4	7.3	7.3	10.4	12.7	5.5	1.8	3.3	4.6
Spirulina platensis	6.7	9.8	7.1	4.8	5.3	5.3	2.5	0.9	0.3	6.2	9.5	7.3	11.8	10.3	5.7	2.2	4.2	5.1
	5.71	9.26	6.45	4.42	4.45	5.26	2.05	0.59	0.06	4.65	8.51	7.09	9.86	13.4	1.1	1.91	3.33	4.59
	5.6	8.6	6.3	4.7	4.4	4	1.9	0.9	1.7	4.5	7.3	6.9	9.5	14.5	5	1.6	3.8	4.4
Spirulina sp.	6.4	13.9	6.6	7.7	6.8	5.7	4.6	1	3.3	4.7	17.9	8.2	8.9	11.6	11	4.7	6.8	6.4
Aphanizomenon flos-aquae	2.9	5.2	3.2	3.2	2.5	–	0.7	0.2	0.7	3.3	4.7	3.8	4.7	7.8	2.9	0.9	2.9	2.9

4.3 Carbohydrates

As long as micro algae are mainly used as protein rich nutrient, the carbohydrate content is of minor importance. (Becker, 2013)

4.4 Fats

The average fat content of micro algae varies between 1% and 40%, where under certain circumstances it can increase until 85% of the weight based on dry matter. Of the different omega-3 fatty acids in algae, the bioactive EPA (C22:5) and DHA (C22:6) have the most important nutritional value. Long chain EPA / DHA omega-3 fatty acid supplementation can be co-preventively en co-therapeutically. Current research proves that long chain omega-3 fatty acids might be beneficial for health and could act as natural drug against a number of severe diseases. Certain micro algae produce high amounts of EPA / DHA levels. Nowadays, organically produced DHA-rich micro algae oil is available. Clinical trials with DHA-rich oil showed comparable effects as with fish oil in case of protection against cardiovascular risk factors due to a decrease of plasma triglycerides and oxidative stress (Becker, 2013).

Table 5: *Fatty acid composition of lipids of different algae species (mg g^{-1} dry weight), (Becker, 2013)*

Fatty acid	**notation**	***Spirulina platensis***	***Scenedes-mus obliquus***	***Chlorella vulgaris***	***Dunaliella bardawil***	***Porphyri-dium cruentum***	***Oocytis sp.***	***Synecho-coccus sp.***	***Tribonema sp.***
Lauric acid	(12:0)	0.04	0.3	–	–	–	–	0.7	–
Myristic acid	(14:0)	0.7	0.6	0.9	–	–	0.2	5.6	1.1
Pentadecanoic acid	(15:0)	traces	–	1.6	–	–	–	–	–
Palmitic acid	(16:0)	45.5	16.0	20.4	41.7	5.9	3.8	3.4	2.5
Palmitoleic acid	(16:1)	9.6	8.0	5.8	7.3	–	1.5	10.8	5.1
Hexadecatetraenic acid	(16:4)	–	26.0	–	3.7	–	–	–	–
Heptadecanoic acid	(17:0)	0.3	–	15.3	2.9	–	–	–	–
Stearic acid	(18:0)	1.3	0.3	15.3	2.9	–	–	0.1	
Oleic acid	(18:1)	3.8	8.0	6.6	8.8	0.1	3.9	–	0.2
Linolic acid	(18:2)	14.5	6.0	1.5	15.1	2.1	6.4	–	0.2
α- Linolenic acid	(18.3)	0.3	28.0	–	20.5	–	8.1	–	–
γ- Linolenic acid	(18.3)	21.1	–	–	–	–	–	–	–
Eicosadienoic acid	(20:2)	–	–	1.5	–	0.3	–	–	–
Eicosanotrienoic acid	(20:3)	0.4	–	20.8	–	–	–	–	–
Arachidonic acid	(20:4)					6.0	0.5	–	–
Docosapentaenoic acid (EPA)	(20:5)					6.1	1.1	–	–
miscellaneous		–	2.5	19.6	–	–	–	–	–

Table 6: *Most important fatty acids in micro algae biomass (mg/100 g) (average of three measurements for each harvested algae cultivation), (Batista et al., 2013)*

notation / fatty acid	***Spirulina Maxima***	***Chlorella vulgaris* (green)**	***Chlorella vulgaris* (orange)**	***Haematococcus pluvialis***	***Diacronema vlkianum***	***Isochrysis galbana***
14:0	9±0.2	124±13	210±1	154±1	2081±38	3272±3
16:0	1078±26	1016±20	5606±1	5977±12	1413±10	2711±6
18:0	32±1	25±1	406±3	603±10	14±1	50±38
other SFA	26±2	88±12	408±61	988±13	78±5	648±51
Σ SFA	1146±24	1254±45	6630±61	7722±1	3586±23	6681±60
16:01	189±5	78±2	38±1	102±2	2425±41	3275±3
18:01	115±4	449±3	9965±133	11125±51	253±5	584±1
other MUFA	10±1	110±21	329±6	1065±3	193±82	354±18
Σ MUFA	402±10	836±23	10733±141	13387±46	3620±128	4213±21
16:04 ω3	4±0.2	165±1	688±2	1160±6	112±20	–
18:03 ω3 (ALA)	40±0.1	661±12	3665±1	3981±2	14±1	421±5
18:04 ω3	2±0.6	–	–	–	1121	–
20:05 ω3 (EPA)	–	19±1	39±1	579±6	3212±57	4875±108
22:06 ω3 (DHA)	–	16±1	80±1	–	836±41	1156±40
other PUFA-ω3	11±6	111±1	308±3	52±10	113±47	10±1
Σ PUFA-ω3	58±35	971±14	4781±2	5770±14	5407±146	6461±153
18:02 ω6	481±31	292±16	1502±1	7844±20	49±5	123±1
18:3 ω6 (GLA)	452±28	112±11	23±1	472±8	112±3	–
20:04 ω6	–	–	12±0.2	292±1	191±1	162±3
22:05 ω6	–	4±1	–	–	976±33	–
other PUFA-ω6	12±0.2	20±1	10±6	159±20	15±23	2116±75
Σ PUFA-ω6	945±59	428±28	1547±7	8767±230	1343±21	2401±76
ω3/ω6	0.1	2.3	3.1	0.7	4.1	2.7
UI (EPA+DHA)	0	0.05	0.03	0.08	1.4	1.41
UI (Total)	10.3	1.24	1.21	1.43	2.4	1.99

4.5 Vitamins

Micro algae form a valuable source of almost all important vitamins, which improves the nutritional value of algae biomass. Besides natural fluctuations due to environmental factors, the treatment after harvest and the method of drying of the algae biomass have a considerable effect on the vitamin content. This is especially valid for the heat instable vitamins B1, B2, C and nicotine acid, of which the concentrations decrease considerably during the drying process. (Becker, 2013)

Table 7: *Vitamin content of different algae species (Becker, 1994) (values in mg kg-1 dry matter)*

Algae species	Vit A	Vit B1	Vit B2	Vit B6	Vit B12[a]	Vit C	Vit E	Niacine	Biotine	Folium acid	Pantotheen acid
Spirulina platensis	840	44	37	3	7	80	120	–	0.3	0.4	13
Aphanizomenon flos-aquae		4.8	57.3	11.1	8	0.7	–	0.1	0.3	1	6.8
Chlorella pyrenoidosa	480	10	36	23	–	–	–	240	0.15	–	20
Scenedesmus quadricauda	554	11.5	27	–	1.1	396	–	108	–	–	46

[a] All data in the literature concerning the content of vitamin B12 in Spirulina sp. have to be interpret very carefully, because large fractions of this vitamin are not-nutritive analogues.(Becker, 2013).

Some *Chlorella* species contain more vitamins than most of cultivated crops. *Spirulina* contains more than ten times higher β-carotene levels compared to any other foodstuff, like carrots, and more vitamin B12 compared to whatever fresh plant or animal food source. Compared to green algae, spinach and the liver, *Spirulina* represents the most rich source of vitamin E, thiamine, cobalamin, biotin and inositol. Several micro algae species produce α-tocopherol (α-T, the organic active form of vitamin E) in very high concentrations. Rodriguez Zavala et al. (2010) experienced that the production of α-tocopherol in the heterotroph cultured micro algae *Euglena gracilis* reached 3.7 ± 0.2 mg/g after 120 hours, which means, comparable to sunflower oil, soya oil, olive oil and maize, which are some of the most common natural sources of vitamin E, indicating an approximately 13, 18, 95 and 56 times higher α-tocopherol productivity, respectively.

In case that the reported high biomass yield of the micro algae *Tetraselmis suecica* can be reached, it can compete with *E. gracilis* as a candidate for commercial α-T production. In the six species of seaweed, which were examined by Rodriguez Bernaldo (2010) the level of folate (folic acid) varied to a maximum of 161.6 mg/100g dry matter (Kovač et al. 2013).

4.6 Minerals, ash and nucleic acid level

Table 8: *Mineral composition of micro algae biomass (average of three measurements for each harvested algae cultivation), (Batista et al., 2013)*

	Spirulina maxima	***Chlorella vulgaris* (green)**	***Chlorella vulgaris* (orange)**	***Haemato-coccus pluvialis***	***Diacronema vlkianum***	***Isochrysis galbana***
N (%)	7.19	6.08	2.02	1.64	6.15	6.33
P (%)	1.29	1.53	1.01	1.31	1.49	2.65
K (%)	2.58	0.98	0.45	0.97	0.72	1.19
Ca (%)	0.91	4.73	0.8	0.25	0.91	0.56
Mg (%)	0.35	1.46	0.18	0.22	0.53	0.96
Na (%)	8.53	0.98	4.84	5.87	1.03	1.6
Cu (mg/kg)	1.1	2.2	1	344	1.9	8.6
Mn (mg/kg)	24.6	471.5	11.7	111.9	2548.7	801
Zn (mg/kg)	3.5	17.5	17.8	232.2	91.3	19.2
Fe (mg/kg)	93.6	166.3	17.2	822.7	208.1	14.6

Ortega-Calvo et al. (1993) analysed three samples of *Spirulina*, *Chlorella* and a triplet of seaweeds. They found a large variation in mineral content, as shown in Table 8. The ash content in the algae species was significantly lower than in the seaweeds, ranging between 7 and 14%.

The nucleic acid content in the algae was higher than in the seaweeds, and amounted about 5%. This variation in nucleic acid content within species is affected by the cultivation circumstances (Marrez et al., 2014). The possible accumulation of metals in the algae is a point of attention. It occurs when they are cultivated under conditions of high metals availability. This is probably also valid for the nucleic acid and ash content.

5 Health promoting properties

5.1 General

Micro algae contain high levels of components, which might have biological activities and unique structures. In the last decades, micro algae, especially cyanobacteria, where screened for their properties as potential medications and antibiotics. Published data until 1996 revealed 208 cyanobacterial substances with biological activity, whereas in 2001 the number of screened substances was increased to 424, including lipoproteins (40%), alkaloids, amines, and others. The reported biological activities consist of cytotoxic, antitumor, antibiotic, antimicrobial (antibacterial, antifungal, antiprotozoal), antiviral (for example anti-HIV) activities, and also immunosuppressive and anti-inflammatory effects. The cytotoxic activity, important for the development of medication against cancer, is probably related to the defending strategies in the firm competitive watery environment, because mostly only those organisms in which an immune system is lacking, are good producers of secondary metabolites, such as toxins. Even a very small amount of micro algae biomass can influence the physiology of animals positively by an improved immune response, which results in improvement of daily gain, resistance against diseases, antiviral and antibacterial effects, improved functioning of intestines, prebiotic effects, whereby the desired bacteria can colonize the intestines, and also by improved feed conversion ratio, reproduction ability and control of weight. Consuming algae also can improve external properties, as expressed by a healthy skin and a shiny coat, both in farm animals (poultry, cows, bulls) and pets (cats, dogs, rabbits, ornamental fish and birds). An extensive number of nutritional and toxicological evaluations have been carried out, which have proven the ability of algae biomass as valuable nutritional supplement (Gouveia et al., 2008).

5.2 Antibacterial characteristics

In the last decade, resistance of pathogens against antibacterials has increased significantly. Pratt et al. (1944) isolated the first antibiotic relationship out of *Chlorella algae*; a mixture of fatty acids, named chlorellin, pointed out to be responsible for the inhibiting activity against both Gram positive as well as Gram negative bacteria.

In the meantime, increased research efforts have been done to identify effective antibacterial components in micro algae. This because of the awareness of risks for insensibility of conventional antibiotics. An example of it is the increased concern in health centres worldwide, because of the increasing numbers of multi resistant *Staphylococcus aureus* (MRSA) tribes. Although micro algae can synthesize a few useful products, research towards new antibiotics is still in its infancy. illustrative examples of possible antibacterial properties of micro algae are shown in Table 9 (Amaro et al, 2011).

Table 9: *Antibacterial characteristics of selected relationships out of micro algae. (Amaro et al, 2011)*

micro alga	**active substance**	**Effective against:**
Phaeodactylum tricornutum	Eicosapentaeen acid	*MRSA,* *Listonella anguillarum,* *Lactococcus garvieae,* *Vibrio spp.*
Haematococcus pluvialis	short chain fatty acids	-
	short chain fatty acids (butane acid and methyl lactate)	*Escherichia coli,* *Staphylococcus aureus*
Skeletonema costatum	unsaturated, saturated long chain fatty acids	*Vibrio spp.*
Euglena viridis	organic extracts	*Pseudomonas,* *Aeromonas,* *Edwardsiella,* *Vibrio,* *E. coli*
Skeletonema costatum	extra-metabolites	*Listeria monocytogenes*
Staurastrum gracile *Pleurastrum terrestre* *Dictyosphaerium pulchellum* *Klebsormidium crenulatum*	methanolic extracts	-
Chlorococcum sp.	watery extracts	
Chlorococcum HS-101	α- linolenic acid	-
Chlorokybus atmophyticus	acetone extract	-
Chlamydomonas reinhardtii	Mehanolic and hexanolic extracts	*Staphylococcus aureus,* *Staphylococcus epidermidis,* *Bacillus subtilis,* *E. coli,* *Salmonella typhi*
Chlorella vulgaris		

5.3 Antiviral characteristics

In the last years, a number of viral infection diseases commenced (and arisen again). Although several antiviral products where developed against this diseases, resistant mutations arise continuously. Therefore, new antiviral active substances are necessary, especially those which are not exposed to viral pools. For this reason, micro algae have received strong attention as potential supplier of antiviral products. Some selected examples are presented in Table 10 (Amaro et al, 2011).

***Table 10:** Antiviral characteristics of selected relationships out of micro algae. (Amaro et al, 2011)*

micro alga	active substance	mechanism of action	Effective against:
Navicula directa	polysaccharide	inhibiting hyaluronidase	HSV1 & 2, Influenza A virus
Gyrodinium impudicum	p-KG03 exopolysaccharide	inhibiting (or slowdown) cytopathic effect	Encephalomyocarditis Virus
Dunaliella primolecta	Pheophorbide α-, β-like substances	inhibiting cytopathic effect	HSV1
Chlorella autotrophica		inhibiting replication *C. autotrophica*: 47.4-67.4 %	
Ellipsoidon sp.	sulphated polysaccharides	*Ellipsoidon* sp.: up to 44 %	VHSV, ASFV
Cryptomonads	allophycocyanin	inhibiting cytopathic effect, slowdown of synthesis viral RNA	Enterovirus 71
Cochlodinium polykrikoide	extracellulair sulphated polysaccharides	inhibiting cytopathic effect	Influenza virus A & B, RSV A & B, HSV-1

Experiments with water soluble extracts of cyanobacteria have proven that a new found sulphated polysaccharide, spirulan calcium (Sa-SP), has an antiviral effect. This substance exists of rhamnose, ribose, mannose, fructose, galactose, xylose, glucose, glucuronic acid, galactoronic acid, sulphate and calcium, whereas is supposed that a chelating of the calcium with sulphate groups is essential for the antiviral effect. This seems to selectively inhibit the penetration of enveloped viruses in host cells selectively, whereby replication is prevented. The effect is described for several viruses, such as Herpes simplex virus type 1, human cytomegalo virus, measles virus, mumps virus, influenza A virus, and even HIV-1. (Becker, 2013)

5.4 Antioxidants

Antioxidants belong to the most important connections in algae. Algae biomass can be considered as a multi component antioxidant system that in general is more effective through the interactions between the different antioxidant components. The most powerful water soluble antioxidants in algae are polyphenols, phycobiliproteins and vitamins. Because they are photo-synthetically organisms, algae are exposed to light and high oxygen concentrations, and in cultures with a high cell density ad in closed photo-bio reactors the oxygen concentrations can become very high. Such circumstances promote the accumulation of very effective anti-oxidative complexes to protect cells. The anti-oxidative characteristics of *Spirulina platensis* for example can increase twice to three times under conditions of oxygen stress. Because of their capacity scavenge free radicals, micro algae are processed in products for functional foods, especially in the drinks market segment (Kovač et al. 2013).

Algae contain pigments like chlorophyll and carotenoids, which are used as pigments in the food, pharmaceutical and cosmetic industry. These pigments can also be applied as antioxidants. An example of this is the substance C-phycocyanin, a protein-bound pigment that is found in blue-green algae. This pigment has shown to have anti-inflammatory and antioxidant characteristics. It is applied as a remedy in case of locomotion disorders in dogs, horses and humans. From a research with lame horses, it was shown that either or not providing C-phycocyanin on a daily base over a one-year period tended to reduce the occurrence of lameness (Taintor et al., 2014).

Chlorophyll
Chlorophyll derivations can promote health. In (the practice of) medicine, these structures are traditionally used because of both their wound healing and anti-inflammatory characteristics. Recent epidemiological studies within the Dutch Cohort Study have found evidence that consumption of chlorophyll resulted in a decreased risk towards colon cancer. The actual nutritional value of chlorophyll in micro algae is quite controversial. In Japan, chlorophyll breakdown products have been indicated as a cause of skin irritations (Becker, 2013).

Carotenoids
All algae contain carotenoids and the variety is bigger than in plants with a higher level of diversification. Foundation of the protecting effect of these connections against/towards oxidative stress in many organisms and situations is the anti-oxidative activity of carotenoids. More and more evidence exists that some carotenoids play an important role in humans and adequate intake prevents degenerative diseases. Therefore, carotenoids are used as pigment in foodstuffs and cosmetics, in vitamin supplements, and in health improving food, but also as feed additive for poultry, livestock, fish and crustaceans (Becker, 2013).

β-carotene is the most important carotenoid, because it is most active as pro-vitamin A, and it is used as a pigment, a pro-vitamin, an additive in multivitamin supplements, and a health promoting nutrient with an antioxidant claim. The natural form of this pigment has a stronger effect than the synthetic one because it is absorbed more easy in the body. Although *Spirulina* is the most familiar nutritional source of this carotenoid, for this reason mainly *Dunaliella salina* is produced at a large scale, with concentrations up to 16% of the dry weight. Products derived from *D. salina* are β-carotene extracts, *Dunaliella* powder for human consumption, or for usage in animal feed, and preparations as a complete source of carotenoid nutrition. Evidence increased that astaxanthin exceeds the antioxidant capacities of β-carotene, vitamin C, vitamin E, and many xanthophylls. This carotenoid is used as pigment in the aquaculture, as well as in nutraceuticals and in the food and feed industry. Although the natural form commercially cannot compete with the synthetic source, it is preferred for some specific applications. *Haematococcus pluvialis* is a rich source for astaxanthin and has been cultivated on large scale. (Kovač et al. 2013)

5.5 Improving the immune system

Several experiments with intact cyanobacteria and water extracts in human, mice, rats, cats and poultry showed significant effects on phagocytosis, NK-cell function and inflammation. Further studies indicate that cyanobacteria can inhibit mast-cell mediated Type I allergic reactions and even anaphylactic reactions in intra peritoneal application in rats. It was demonstrated that *Spirulina*-

extracts resulted in a decrease of anaphylactic mortality, inhibition of local intolerance reactions and reduction of serum histamine levels. (Becker, 2013)

Pugh et al. (2001) conducted experiments with Immulina, a polysaccharide extract out of *Spirulina* with a high molecule weight. They observed that this extract is 100 up to 1000 times more active in the activation of monocytes in vitro than polysaccharides preparations which were used at that time in clinical centres for immune therapy on cancer. This 100 up to 1000-fold increase in activity is quite remarkable. They continued with two more studies in 2006, one in vitro and one in rodents. Both studies confirmed their previous conclusions concerning the improved effect of the immune system.

From a Korean report it points out to be that *Spirulina* prevents atherosclerosis by decreasing hypercholesterolemia in rabbits which were fed a high cholesterol diet (HCD) during 4 weeks and subsequently during 8 more weeks HCD complemented with 1% or 5% *Spirulina*. *Spirulina* supplementation decreased the intimal surface of the aorta with 32.2-48.3%; serum triglyceride (TG) and total cholesterol (TC) were reduced dramatically. Serum-LDL-C decreased remarkably with 26.4% in 1% and 41.2% in 5% *Spirulina,* respectively. On the other hand, HDL-C increased considerably in both groups of algae. Consequently, *Spirulina* can be useful in preventing atherosclerosis and reducing risk factors towards cardiovascular diseases (Becker, 2013).

6 Research results per animal category

6.1 Poultry

Partial protein replacement
Up to an inclusion level of about 5-10%, algae can be safely used as partial replacers of conventional protein sources of the poultry diet. Providing higher concentrations might in the long-term cause adverse effects, like a less efficient feed conversion and a decreased protein and energy efficiency. (Becker, 2013)

Photo 4: White Leghorns

In laying hens, no differences were observed in egg production and egg quality (size, weight, shell thickness, solid content of the egg, albumin index, etc.), and feed conversion ratio between hens fed diets containing 12% *Chlorella* (cultivated on effluent) and hens fed the control group (Gouveia et al., 2008). Ekmay et al. (2015) found comparable results with a diet with 25% defattened *Desmodesmus.* They report also an improved hen health. A considerably increased daily gain was observed in turkeys fed a diet with 1-10 g per kg *Spirulina* (Becker, 2013).

The effect of sun dried *Spirulina platensis* in poultry diets was examined during a 12 week study, in which in a commercial diet fish meal or peanut cake was replaced by algae in concentrations of 140 and 170 g/kg. No additional vitamins and minerals were added in the algae diets, because *Spirulina* contains a lot of them already. Based on a similar feed efficiency, protein conversion rate, and the extent of lean meat, it was concluded that substitution of fish meal or peanut cake by algae did not affect broiler performance. Moreover, none of the diets affected body weights, body composition and histopathology of the various organs of the broilers. Meat quality remained also unchanged. The only effect of the supplementation of algae was a more intensive colouring of the broilers (Venkataraman et al., 1994).

Body weights of broilers fed *Spirulina* concentrations of 11.1% and 16.6% of the diet did not differ from those in the control group, fed with peanut cake.

Broilers in another study fed *Spirulina* concentrations of 0, 40 or 80 g per kg diet during 16 days did not significantly differ in body weight.

Broilers fed *Spirulina* concentrations of 40 g per kg diet, however, had a more intense redness and yellowness in the muscles compared to the control group.

White Leghorn pullets fed *Spirulina* concentrations of 0, 0.001, 0.1, 1 and 10 g per kg had comparable body weights after feeding the diets for 7 weeks. (Holman & Malau-Aduli, 2013)

Egg yolk quality improvement

Laying hens that will be fed special micro algae (like heterotrophic growing *Schizochytrium* and *Cryptecodinium*) might produce "OMEGA" eggs. Therefore, by manipulating animal diets, food can be produced that might influence human health in a positive way (Pulz & Gross, 2004).

Dunalliella Bardawil is a source of vitamin A and a yolk colour improving feed additive for laying hens. It has been reported that the effect of carotenoids, extracted out of *Chlorella vulgaris* micro algae biomass, on the pigmentation of the egg yolk is comparable with commercial synthetic pigments. *Haematococcus* micro-alga can also be used as a natural pigment in broiler diets. Laying hens, fed the red micro-alga *Porphyridium sp*. biomass (5% and 10% of the diet), had a decreased cholesterol level and a changed fatty acid composition in the egg yolk, whereas body weight, number of eggs and egg weight were not affected. Both groups of algae fed hens consumed 10% less feed, whereas their serum cholesterol levels were 11% and 28%, respectively, lower compared to the control group. In another study, laying hens fed diets supplemented with 5% algae had decreased cholesterol levels and increased linoleic acid and arachidonic acid levels (29% en 24%, respectively) in their egg yolk. Yolk colour was darker as a result of the 2.4 times higher carotenoid concentrations in the algae fed hens (Gouveia et al., 2008).

Supplementing laying hen diets with saltwater algae (up to 4.8%) increased DHA and decreased n-6 fatty acid contents in the eggs. Neither the egg production nor other egg quality parameters were affected by dietary algae supplementation in the 56 weeks old hens. Dietary addition of fermented *Schizochytrum sp.* (up to 4.3%) to laying hen diet improved egg production and feed efficiency. Supplementation of 0.86% and 4.3% micro algae increased the DHA content in the egg with 134 en 220 mg per egg, respectively (Lum et al., 2013).

In white leghorns, total cholesterol level decreased compared to the control group when the diet was supplemented with 150 g linseed + 200 mg vitamin E + 3 g *Spirulina* per kg (Holman & Malau-Aduli, 2013). In 32 wk old white leghorns, that were fed 20% linseed and 5% *Spirulina* (w/w), egg production and levels of linoleic acid were increased, cholesterol level was decreased, where egg yolks were darker compared to the control group. Optimal egg yolk pigmentation was obtained after feeding a diet supplemented with 1% *Spirulina*, compared to a feeding a diet without xanthophylls. Egg yolk carotenoid pigments and omega-3 fatty acids increased when white leghorns were fed 150 g linseed + 200 mg vitamin E + 3 g *Spirulina* per kg feed (Holman & Malau-Aduli, 2013).

Immune response improvement

White Leghorns (Cornell K) and broilers that were fed diets with different levels of *Spirulina platensis* (0–10 g kg^{-1}) from hatch to 7 and 3 weeks of age, respectively, had improved immune functions, as shown by an increased:

- SRBC antibody response
- PHA-P mediated lymphoproliferative response
- phagocytosis activity of macrophages
- NK cell activity

(Qureshi et al., 1996).

A study was carried out to determine the effects of different levels of micro algae *Chlorella sp.* on the performance of laying hens under heat stress. *Chlorella* micro algae did not influence the performance (egg production and egg weight, feed intake and feed conversion ratio). Addition of *Chlorella* to drinking water improved the immune response (higher SRBC antibody response) (Moradi en Mohamadi, 2014).

Chickens fed 10 g per kg *Spirulina* had a higher NK cell activity compared to the control group, indicating an increased immunity. Supplementing incremental *Spirulina* levels (0.5%, 1% en 2%) to the diet linearly improved phagocytosis activity in hens (Holman & Malau-Aduli, 2013).

Broilers fed a diet with 1% *Chlorella vulgaris* during the first 4 weeks of life had in this period a 3.5% higher body weight gain (1549 vs. 1603 g) and a 8.5% better feed efficiency (FCR decreased from 1.66 up to 1.52) (Kang et al., 2013). These changes coincided with a higher concentration of immune globulins (IGA and IGM) in the blood plasma.

In turkeys fed *Spirulina* (1-10 g per kg feed), a lower nonspecific mortality was observed. The mortality decreased from 12% in birds receiving the control diet to 3% when 1 g algae per kg diet was added (Becker, 2013).

6.2 Pigs

Protein replacement

Next to poultry, pigs also seem a potential animal category in which algae can be used as nutritional supplement. Dietary supplementation of *Chlorella* and *Scenedesmus,* as replacers of soy bean meal and cotton seed in concentration up to 10%, did not affect feed conversion ratios. In general, biomass of micro algae can be considered as a feed ingredient with a proper nutritional quality. It can replace conventional proteins like soy bean meal or fish meal, while no adverse effects of algae on feed intake are reported in this animals. *Spirulina* also has been tested as feed additive in short and long term experiments and all studied parameters remained unchanged, and also no effects on reproductive parameters were observed. A share of 25% micro algae biomass in the diet is recommended, whereas other authors assumed that a dietary portion of 33% will negatively affect performance of the pigs (Gouveia et al., 2008).

Photo 5: Piglets

Daily gain improvement

Holman & Malau-Aduli (2013) summarized the following results, based on several different studies:

Daily gain of weaned piglets, fed diets supplemented with 1.5% and 3% *Spirulina*, was increased compared to the unsupplemented control group.

Weaned piglets fed pelleted feed enriched which *Spirulina* showed a decreased average daily gain, while piglets fed the same diet as meal had an increased average daily gain.

The average daily gain of pigs fed diets supplemented with 2% *Spirulina* from day 14 to day 28 after weaning was increased compared to the control group.

Pigs fed diets with an inclusion level of 14% *Spirulina* showed a comparable daily gain as pigs fed diets supplemented with lean milk powder.

Providing diets with graded levels of *Spirulina* (0.5%, 1% and 2% of the diet) to pigs only numerically increased average daily gain.

The summarized results were not very conclusive. Possibly, there might be an effect of age of the pigs. Effects seem to be more positive in weaned piglets compared to older pigs.

As shown in the table below, the final weight and average daily gain of piglets fed *Chlorella* was at the end of the weaning period significantly higher in two out of four trials conducted at the Regional Research Centre in Germany (Weber and Grimmer, 2001 in Pulz and Gross, 2004).

Results of Chlorella feeding trials with sows and piglets at the Regional Research Centre (LVA; Iden, Germany; Weber and Grimmer 2001, in Pulz and Gross, 2004)

Parameter	Trial 1		Trial 2		Trial 3		Trial 4	
	Control	Algae	Control	Algae	Control	Algae	Control	Algae
Results during lactation								
Daily gain (g/d)	290	305	319	318	303	300	304	308
Weight at weaning (kg)	7.5	7.9	8.5	8.5	7.2	7.18	7.8	7.8
Weight end weaning phase (kg)	23.8	24.9	26.9[a]	29.8[b]	24.5	25.7	25.1[a]	26.8[b]
Period from weaning until end of weaning phase (d)	42	42	46.2	45.8	47	46.1	45	44.6
Results during weaning phase								
Daily gain (g/d)	388	404	396[a]	466[b]	369	403	386[a]	424[b]
Feed conversion ratio (kg/kg)	1.67	1.66	1.74	1.66	1.73	1.57	1.71	1.63
Mortality (number)	0	0	1	0	3	0	4	0

[a,b] Level of significance $P<0.05$

Improvement of meat quality
Daily gain of pigs, that were fed an iodine rich algae species, increased by 10%, but also iodine content in meat increased, indicating that this type of algae might be used for production of iodine rich pork for human consumption.

In pigs fed micro algae, increased DHA concentrations in loins and subcutaneous fat were observed. This increase, however, did not show a doses dependent response (Lum et al., 2013). This study did not provide the algae inclusion level for obtaining the maximum effect on DHA content in the measured tissues.

Fertility improvement of boars
Boars fed 1.5 ml BioR (originating from *Spirulina*) per day had a bigger ejaculation volume and a greater spermatozoa mobility compared to a control group (Holman & Malau-Aduli, 2013).

6.3 Dairy cows

Milkproduction and milk quality improvement
The effect of *Spirulina platensis* on milk production and serological parameters in dairy cows was studied by Simkus et al. (2007). A control diet without *Spirulina* was compared with an experimental diet, providing 2 g fresh *Spirulina* per cow per day. Cows which received *Spirulina* during the 60 days experimental period showed a 7.6% (136 kg) increase in average milk yield compared to cows fed the control diet. In cows

receiving algae the average amount of milk fat was increased with 17.6-25%, the average milk protein with 9.7%, and the amount of lactose with 11.7% ($P<0.001$) compared to the control group. Besides, the addition of *Spirulina platensis* decreased the amount of somatic cells in milk with 29.1%. These results seem very unlikely, because it seems rather unrealistic that feeding 2 g wet algae per day resulted in an increased daily milk production of more than 2 l.

A comparable experiment, but with a much higher dietary algae concentration, is described by Kulpys et al. These authors tested the effect of *Arthrospira platensis* (*Spirulina*) biomass additives on the physical condition of milk producing cows, and the biochemical milk parameter were examined during a 90 days experiment, in which 200 g of algae was administered per cow on a daily base. Cows in the experimental group became 8.5–11% more fat. Average milk production at the start of the lactation period was in the experimental group 34 kg per day, which was over 6 kg more than the control group. (Becker, 2013)

Photo 6: Dairy cows

In Holstein crossbreds fed 40 g *Spirulina* per day, the saturated milk fat acids decreased, whereas mono- and polyunsaturated fatty acids increased. (Holman & Malau-Aduli, 2013)

Micro algae biomass or oil can be added in the diet of ruminants to improve their milk fatty acid composition. The level of n-3-fatty acids in milk, especially DHA, pointed out to increase by intake of algae biomass or oil, without influencing milk production.

However, a study with comparable algae intake in the diet led to a decrease of the milk fat level.

Many studies in ruminants were conducted aiming to increase the n-3 fatty acid content in milk. In such a study of Stamey et al. (2012), either biomass of algae or oil was fed to dairy cows. The total amount of milk fat and the yield were not affected by supplementation of algae, but both interventions increased the milk DHA (C22:6) content. Glover et al. (2012) indicated that a diet containing micro algae and fresh crops resulted in a decrease of the total milk fat level, but in an increase of the DHA concentration. A parallel study was conducted in sheep to determine whether supplementation with algae and (or) also with sunflower oil in the diet improved the nutrient profile of milk. The milk yield was not influenced by the treatments, whereas the milk fat level decreased and the milk DHA concentration increased due to the increase of the dietary algae level. Franklin et al. (1999) experienced that feeding algae to dairy cows either protected against ruminal bio hydrogenation or increased the milk DHA concentration, without influencing milk fat level. In summary, supplementation of micro algae to diets of dairy cows consistently resulted in increased concentration of n-3 PUFA in milk, with variable effects on milk fat level (Lum et al, 2013).

6.4 Veal calves

Cholesterol decrease
An Iranian study investigated the performance, digestibility, and serum parameters in veal calves fed diets differing in the amount of *Spirulina.* The experiment lasted 57 days and the daily amount of administered algae was 2, 6 and 25 g. The treatments did not significantly influence final body weight, daily gain, daily feed intake, and feed efficiency, whereas increased supplementation of *Spirulina* up to 25 g resulted in a decreased digestibility of dry matter, crude protein, fibres (NDF) and organic matter. A significant decrease of the plasma cholesterol concentration was found in animals fed the 25 g *Spirulina* compared to the control groups (Becker, 2013).

6.5 Lambs

Improvement of daily gain in lambs
Six month old lambs fed with 10% (w/w) *Spirulina* showed a higher live weight than lambs fed 20% (w/w) and the control group.

Spirulina had a dose-dependent improvement of the physical condition of the lambs at supplementation levels of 10% and 20% (w/w) compared to the control group.

Lambs fed cow milk enriched with 10 g *Spirulina* per day at 15-30 days of age showed a higher daily gain than the control group.

Pregnant ewes fed ad libitum with feed pellets containing 2 g *Spirulina* gave (birth to) lambs with higher birth weights and a higher daily gain than those of ewes fed the control diet. (Holman & Malau-Aduli, 2013)

6.6 Fish

A research with carps pointed out that *Spirulina* is well able to replace part of the fish meal in the diet, without negatively affecting the performance or fish quality (Abdulrahman and Hamad Ameen, 2014).

7 Economic aspects of algae in livestock diets

Algae could be used as substitute of protein in livestock diets, as source of poly unsaturated fatty acids (PUFA) or as feed additive with health improving characteristics. The currently relatively high cost-price of algae is a limiting factor for feed applications.

7.1 Cost-price of algae

Currently, the cost-price of algae is relatively high compared with the conventional protein sources, e.g. soybean meal. In particular, the costs for construction and installation of the basin or the photo bio reactors are high, together with the costs of electricity. By scaling up, the relative costs for the installation can be substantially reduced, but the costs of electricity still remain high, particularly to keep circulating the algae-water (Spruijt et al., 2014). Also, a lot of energy is required for the post-harvest processes which are necessary for further processing of the algae, e.g. drying, before application in livestock diets.

Based on the chemical composition and the *in vitro* digestibility an estimation of the nutritional value of dried algae was made, after which a growing finishing pig diet was optimized including these algae (Van Krimpen et al., 2014). These calculations show that algae up to a cost-price of € 0,30 per kg can compete well with other livestock feed resources. At this price level, about 5% dried algae is added to the diet. The minimum cost-price at large-scale production of algae, however, still amounts € 5 per kg (Spruijt et al., 2014).

By using a poultry farm model (Bedrijfswijzer Pluimvee; Vermeij and Kanis, 2005) the economic value of the realised improvements in performance in the broiler experiment of Kang et al. (2013) was calculated. In this experiment, daily gain increased with 3.5% and feed conversion ratio improved with 8.5%. Based on the standard prices in the poultry farm model the control group realized a gross margin of € 31.25 per 100 broilers, whereas the gross margin in the *Chlorella* group was € 41.12 under these assuptions. At a cost-price level of € 5.25/kg *Chlorella,* both groups realized the same gross margin. This means that, based on the improvements of performance, *Chlorella* may cost at maximum € 5.25/kg. In these calculations other possible health effects, like reduced mortality levels and less use of antibiotics, are not included in this estimation.

7.2 Protein replacement

Nowadays, soybean meal is an important protein source in livestock diets, because of the very high protein content and protein digestibility, the well-balanced amino acid profile, and the low cost-price. Especially in pig and poultry diets, high percentages of soybean meal are included. Soybean meal replacers only have the possibility to be included in a diet if they combine a high protein value with an attractive price (Kamp et al., 2008). Algae will have to compete with imported soybean meal in The Netherlands (mainly from Argentina and Brazil).

Foto 7: Soya beans

Currently, the cost-price of algae is relatively high compared to other protein sources. Because of this, large-scale use in livestock diets is not obvious. Moreover, taste and odour sometimes limit application in animal nutrition. Production costs for algae, however, can be decreased by the development of cheaper growth substrates for the algae, a more efficient use of nutrients by the algae, an increased growth, and a further innovation in the culture, harvest and drying methods. In addition, research towards marketing methods, and the influence of this on product quality expands, thereby gaining more insight in the practical aspects of for instance application of *Spirulina* (Holman & Malau-Aduli, 2013).

7.3 PUFA's

Promising applications of algae lipids always have to be considered realistically against the background of the current economic situation. Considering the actual world market prices for salmon oil (14% EPA, 12.4% DHA, 2.8% Docosapentaeen acid) and fish oil (18% EPA and 12% DHA) algae lipids under present circumstances cannot compete with the conventional sources of PUFA's, even if a higher price is received for these lipids because they are plant-based (Becker, 2013). However, in kitchens of health care centres the usage of fish is not preferable, and because of this the diets of inhabitants of these care centres often contains insufficient n-3 fatty acids. Enrichment of meat or eggs with n-3 fatty acids by feeding pigs and poultry algae-rich diets can contribute to solve this problem.

7.4 Feed additives

Market perspectives for the application of micro algae as feed additives are positive because of the different positive effects on animal health. Furthermore, the fact that several countries want to diminish the addition of antibiotics in feed is an incentive to use other health improving feed additives (Voort et al., 2014).

8 Algae culture and sustainability

8.1 Less land use

Micro algae biotechnology is comparable with conventional agriculture, but has a much higher production potential than the traditional crops and can be transferred to areas and climates which are unsuitable for agricultural purposes (like desert and coastal environments) (Gouveia et al., 2008).

Algae can produce higher amounts of proteins per hectare of land than the current plant crops, and also fixate more nitrogen per surface unit. In open basins with a light efficiency of 1.5%, a water temperature of 25°C, and under Dutch daylight conditions algae currently produce 15 tons of dry weight per hectare, but in theory they could at least produce 25 tons of dry weight per hectare. In case of a protein content in the harvested algae of about 50%, this potentially result in a protein yield of 12.5 tons per hectare. The protein yield of plant crops is about 1-2 tons per hectare (Van der Weide et al., 2014).

Although 71% of the world consists of water, the production capacity possibilities of the oceans, are hardly utilized. Seaweeds and micro algae are also very effective bio filters, which are able to reduce polluting components from the environment (Rabbinge, 2013).

8.2 Recycling of nutrients

Micro algae can effectively eliminate nutrients out of water, e.g. nitrogen and phosphorus (Gouveia et al., 2008).

On the algae production sites at ACRRES-Wageningen UR and Kelstein, rest sources are re-used. At both locations a biogas installation is available with a link to a CHP (Combined Heat and Power) device. Because of this, electricity, residual heat, and released CO_2 will be used together with other nutrients for algae cultivation in ponds and reactors.

At the ACRRES site, research is conducted in cooperation with Algae Food & Fuel, towards among others the purification of effluent with algae in LED-reactors. Presently, for the cultivation of algae still a lot of electricity is needed, certainly when LED-light has been used.

At the veal farm of Kroes in Uddel (The Netherlands) manure is fermented, after which the digested product digestate is subdivided into a thick and a thin fraction. The thin fraction of the digested product will be applied for the cultivation of duckweed. For the cultivation of duckweed. Eventually, also electricity and residual heath produced by the

CHP device, which is linked to the fermenter, will be used. The duckweed is harvested and directly fed to the veal calves. The dirty water, that remains after a period of harvesting the duckweed, and eventually the veal urine, is cleaned by cultivating algae on it. Besides the dirty water and urine, also electricity, residual heath and flue gasses out of the CHP are used for algae production. The watery algae mass is used for providing the water supply of the veal, whereas the algae has a nutritional value as well.

8.3 Declining energy consumption and greenhouse gas emissions

The import of protein containing resources in The Netherlands causes a lot of transport movements, and by this fossil energy use, CO_2 emission, airborne particulates emission, and use of space for roads. Production of proteins in The Netherlands definitely results in the least transport movements (Kamp et al., 2008).

The production of micro algae is an important natural way of reducing the excess of CO_2 in the atmosphere, because of bio fixation and recycling of bounded carbon out of products, consequently resulting in decreased greenhouse gas emissions, global warming, and climate changes (Gouveia et al., 2008).

Currently, the cultivation of algae requires a lot of electricity, mainly because of pumping around the algae water. Also for drying algae to produce a product that can be used for livestock feed applications, a lot of energy is needed.

8.4 Improvement of (phosphate) cycles

Phosphate is a non-renewable resource. Once dissolved in (sea) water, at present it is not economically responsible to regain it. This is an important item, because it is expected that the global phosphorus stocks are running out between 50 and 100 years. Algae are able to recycle phosphate and other nutrients out of a watery environment, and with this it will be possible to create a better local closed-loop system and to limit losses of these nutrients. By local production of algae as protein source, the import of soya can be partly restricted, which simultaneously might imply a limitation of transport kilometres and transport of minerals from one continent to the other.

8.5 No extinction of endangered fish species

Currently, EPA and DHA particularly are gained out of fatty fish, whereas many fish species are critically endangered. Gaining EPA and DHA out of algae does not provide any risk for the endangered fish species.

9 Opportunities

9.1 Algae as bulk application in livestock diets

- On a dry matter base, algae contain comparable or even higher levels of crude protein, carbohydrates and fats than conventional resources (e.g. soybeans). There is quite a lot of variation in nutrient composition among the different micro algae, but:
  - most algae have a **high protein level**. As shown in literature, algae can be supplemented to diets of growing finishing pigs at levels up to 14%, and possibly even up to 33% without having negative effects on performance. In laying hens and broilers the addition of 12% and 17% algae, respectively, pointed out to be quite feasible without influencing performance in a negative way.
  - various algae have a **high fat level** ad a **high content of polyunsaturated fatty acids**, among whom **EPA and DHA**.
  - most algae have a **high level of essential vitamins**

9.2 Algae as livestock feed additive

- There are indications from literature that algae have **health promoting** properties:
  - The **antibacterial characteristics** of various algae species have been proved. Therefore, these algae might contribute in decreasing the use of antibiotics in livestock, and as a consequence the resistance of bacteria against these antibiotics both in human and livestock.
  - Also some studies showed the **antiviral** c**haracteristics** of algae.
  - Algae might contain several types of **antioxidants** (the pigments **chlorofyl** and **carotenoids**), which have **anti-inflammatory characteristics** and might **prevent degenerative diseases**.
  - Algae could promote **immune responses,** which might prevent severe disease phenotypes in case of infections, thereby maintaining the levels of daily gain, feed conversion ratio, reproduction capacity and external characteristics like a healthy skin and a shiny coat. Such effects are shown in studies with in **poultry, sows and piglets, ewes and lambs, and dairy cows**.
  - Supplementation certain types of algae to laying hens, broilers or dairy cows resulted in increased levels of **polyunsaturated fatty acids** in **eggs, meat and milk**, respectively.
  - **Veal c**alves that were fed algae supplemented diets had **reduced cholesterol levels.**
  - The pigment **carotenoid** in algae stimulates the **pigmentation of the egg yolk** and the **colour of roasted chickens.**

- Several algae (especially micro algae) have **high contents of essential minerals.**

9.3 Algae in livestock feed and sustainability

- Application of algae in livestock feed can contribute to sustainability because:
    - For the production of the same amount of protein **land use is substantial lower** compared to traditional protein crops.
    - Algae production **does not compete with agricultural land** so no replacement of current crop cultivation is needed.
    - Algae are able to convert rest sources like **CO_2, residual heath, and nutrients in manure and waste-water (nitrogen and phosphate)** into valuable products.
    - Algae can contribute to **CO_2 uptake** from the atmosphere, thereby reducing the amount of greenhouse gasses.
    - In case of local production of algae as protein source, import of soybean meal can be limited, which can involve a **restriction of transport kilometres.**
    - Extraction of EPA and DHA out of algae instead of fatty fish **does not increase risk levels for endangered fish species.**

10 Challenges

10.1 Further prove nutritional value and health aspects

Currently, the cost-price of algae as an energy and protein source still is too high to compete with other compound feed stuffs. Therefore, more research is required to proof the health promoting properties of algae, as well as their positive effects on quality of meat, milk and eggs, because these properties considerably increase the economic value of algae.

10.2 Increase algae yield

Increasing the algae yield per hectare requires applied research. New algae cultivation systems, like cultivation under LED-light or in plastic bags, and new harvest and processing systems, have to be tested in applied research facilities to receive more insight into the possibilities to increase the production capacity.

10.3 Reduction of the energy use

In the different algae cultivation systems, especially for pumping algae water around, still a lot of energy is required. Also drying algae for further processing in livestock feed costs a high amount of energy. More research have to be focused on the possibilities to reduce the energy use for cultivation, harvesting and processing of the algae.

10.4 Quality improvement

More insight has to be obtained regarding the effects of cultivation, harvesting, after-harvesting drying technics and possible further refining steps on nutritional value and active components of algae. For example, research could be focussed on increasing oxidative characteristics and valuable components of algae under stress conditions and/or through specific lighting programs.

10.5 Decrease of cost-price

To be able to compete with soybean meal as protein source, with fish oil as PUFA source, and with other livestock feed additives, the cost-price of algae must be decreased. Development of innovative, more productive algae cultivation systems, with limited costs for equipment and energy use should enable this.

10.6 Risk analysis using rest sources

To be able to use manure, digestate, waste-water, or other rest sources for the cultivation of algae, proper risk analysis have to be made to ensure a safe use of algae in livestock feed.

10.7 Reduction of contaminants

The possible accumulation of heavy metals in algae, if they grow in an environment with a lot of these metals, and possibly also the nucleic acid and ash contents are areas of concern. It is unclear to what extent nucleic acids should be considered as a harmful substance for animals. In a piglet study it was found that nucleic acids had a positive effect on IgA content in the blood plasma, and thereby on the humoral immunity of animals (Sauer et al., 2012). This is in line with studies in broilers, in which was proved that application of algae in the diet also increased the IgA- content in the blood plasma (Kang et al., 2013).

11 Literature

Abdulrahman, N. M., and H. J. Hamad Ameen. Replacement of fishmeal with microalgae spirulina on common carp weight gain, meat and sensitive composition and survival. Pakistan Journal of Nutrition 2014 13(2):93-98.

Amaro, H.M., Guedes, C., Malcata, F.X. Antimicrobial activities of microalgae: an invited review. Science against microbial pathogens: communicating current research and technological advances. FORMATEX 2011.

Batista, A.P., Gouveia, L., Bandarra, N.M., Franco, J.M., Raymundo, A. Comparison of microalgal biomass profiles as novel functional ingredient for food products. Algal Research 2, 164–173, 2013.

Becker, E.W. Microalgae for Human and Animal Nutrition. Handbook of Microalgal Culture: Applied Phycology and Biotechnology , Chapter 25. 2013.

Chisti Y: Biodiesel from microalgae. Biotechnol Adv 2007, 25:294–306.

Ekmay, R.D., Chou, K., Magnuson, A. and Lei, X.G. Continual feeding of two types of microalgal biomass affected protein digestion and metabolism in laying hens Journal of animal science 2015; 93; 287-297

Fevrier C, Seve B: Incorporation of a spiruline (*spirulina maxima*) in swine food. Ann Nutr Aliment 1975, 29:625–650.

Franklin, S.T., Martin, K.R., Baer, R.J., Schingoethe, D.J., and A.R. Hippen. Dietary marine algae (Schizochytrium sp.) increases concentrations of conjugated linoleic, docosahexaenoic and transvaccenic acids in milk of dairy cows. Journal of Nutrition 1999 129(11): 2048-2054.

Gatrell, S. K., M. L. Manor, and X. G. Lei. Developing omega-3 fatty acids-enriched animal products by feeding defatted microalgal biomass from biofuel production. C. U. Department of Animal Science ed. 2014.

Glover, K. E., Budge, S., Rose, M., Rupasinghe, H.P.V., MacLaren, L., Green-Johnson, J., and A.H. Fredeen. Effect of feeding fresh forage and marine algae on the fatty acid composition and oxidation of milk and butter. Journal of Dairy Science 2012 95 (6): 2797-2809.

Gouveia, L., Batista, A.P., Sousa, I., Raymundo, A. and Bandarra, N. M. Microalgae in Novel Food Products. Food Chemistry Research Developments, 2008.

Holman, B. W. B. and Malau-Aduli, A. E. O. *Spirulina* as a livestock supplement and animal feed. Journal of Animal Physiology and Animal Nutrition 97 (2013) 615–623.

Kamp, J., Berkum, S. van, Laar, H. van, Sukkel, W., Timmer, R., Voort, M. van der. Perspectieven van sojavervanging in voer; Op zoek naar Europese alternatieven voor soja. Praktijkonderzoek Plant & Omgeving, 2008.

Kang, H. K., H. M. Salim, N. Akter, D. W. Kim, J. H. Kim, H. T. Bang, M. J. Kim, J. C. Na, J. Hwangbo, H. C. Choi, and O. S. Suh. Effect of various forms of dietary chlorella supplementation on growth performance, immune characteristics, and intestinal microflora population of broiler chickens. J. Appl. Poult. Res. 2013 22(1):100-108.

Kovač, D.J., Simeunović, J.B., Babić, O.B., Mišan, A.C., Milovanović, I.L. Algae in Food and Feed. Food and Feed Research 40 (1), 21-31, 2013.

Krimpen, M.M. van, Wikselaar, P.G. van, Bikker, P. De in vitro verteerbaarheid van gedroogde algen. Livestock Research Wageningen UR, 2014.

Lum, K.K., Kim, J. and Lei, X. G. Dual potential of microalgae as a sustainable biofuel feedstock and animal feed. Journal of Animal Science and Biotechnology 2013, 4:53

Marrez, D.A., Naguib, M.M., Sultan, Y.Y, Daw, Z.Y. & Higazy, A.M. Evaluation of chemical composition for *Spirulina platensis* in different Culture Media, Research Journal of Pharmaceutical, Biological and chemical sciences, 5(4): 1161-1171, 2014

Moradi kor, N., Mohamadi, N. The Effects of different levels *Chlorella* Microalgae on performance and immune response of laying hens under heat stress condition. International journal of Advanced Biological and Biomedical Research Volume 2, Issue 6, 2014: 2127-2133.

Mostafa, S.S.M. Microalgal Biotechnology: Prospects and Applications. Plant Science Chapter 12. InTech 2012.

Ortega-Calvo, J., C. Mazuelos, B. Hermosin & Saiz-Jimenez, C. Chemical composition of *Spirulina* and eukaryotic algae food products marketed in Spain. Journal of Apllied Phycology 5: 425-435, 1993.

Pratt R., Daniels T.C., Eiler, J.J., Gunninson, J.B., Kumler, W.D., Oneto, J.F., Strait, L.A., Spoehr, H.A., Hardin, G.J., Millner, H.W., Smith, J.H.C., and Strain, H.H. Chlorellin and antibacterial substance from *Chlorella.* Science, 1944, 351.

Pulz, O. and Gross, W. Valuable products from biotechnology of microalgae. Applied Microbiology Biotechnology (2004) 65: 635–648

Qureshi, M.A., Garlich, J. & Kidd, M. Dietary *Spirulina platensis* enhances humoral and cell-mediated immune function in chickens. Immunopharmacol. Immunotoxicol.1996, 18: 465–476.

Rabbinge, prof. dr ir R., Emeritus universiteitshoogleraar Duurzame ontwikkeling en voedselzekerheid. Naar een duurzame, groene economie. Lustrumconferentie van het Application Centre for Renewable RESources (ACRRES) op 7 maart 2013 te Lelystad.

Rodriguez Zavala, J.S., Ortiz Cruz, M.A., Mendoza Hernandez, G., and Moreno-Sanchez, R. (2010). Increased synthesis of α tocopherol, paramylon and tyrosine by Euglena gracilis under conditions of high biomass production. Journal of Applied Microbiology, 109, 2160-2172.

ALGAE IN FOOD AND FEED. Available from: https://www.researchgate.net/publication/281060852_ALGAE_IN_FOOD_AND_FEED [accessed Oct 13, 2015].

Sauer, N., M. Eklund, E. Bauer, M. G. Ganzle, C. J. Field, R. T. Zijlstra, and R. Mosenthin. The effects of pure nucleotides on performance, humoral immunity, gut structure and numbers of intestinal bacteria of newly weaned pigs. J. Anim. Sci. 2012, 90(9):3126-3134.

Spolaore,P., Joannis-Cassan, C., Duran,E and Isambert, A. Commercial Applications of Microalgae. Journal of Bioscience and Engineering Vol. 101, No. 2, 87–96. 2006.

Spruijt, J.; Schipperus, R.; Kootstra, M.; Visser, C.L.M. de; Parker, B. AlgaEconomics: bio-economic production models of micro-algae and downstream processing to produce bio energy carriers, Public Output report WP2A7.10 of the EnAlgae project, Swansea, 2014.

Pugh, N., S.A. Ross, H.N. ElSohly, M.A. ElSohly, and D.S. Pasco. Isolation of three high molecular weight polysaccharide preparations with potent immunostimulatory activity from Spirulina platensis, aphanizomenon flos-aquae and Chlorella pyrenoidosa. Planta Med. 2001 Nov; 67(8):737-42.

Simkus, A., Oberauskas, V., Laugalis, J. Zelvyte, R., Monkeviciene, I., Sederevicius, A., Simkiene, A ., and Pauliukas, K. The effect of weed Spirulina platensis on the milk production in cows. Veterinarija ir Zootechnika 2007 38(60):74-77.

Stamey, J. A., Shepherd, D. M., de Veth, M. J., and B.A. Corl. Use of algae or algal oil rich in n-3 fatty acids as a feed supplement for dairy cattle. Journal of Dairy Science 2012 95(9): 5269-5275.

Taintor, J. S., J. Wright, F. Caldwell, B. Dymond, and J. Schumacher. Efficacy of an extract of blue-green algae in amelioration of lameness caused by degenerative joint disease in the horse. Journal of Equine Veterinary Science 2014 34(10):1197-1200.

Van Kasteren, J. Ecoferm! De kringloopboederij. Innovatienetwerk Rapportnr. 11.2.248, 114 p., 2011.

Venkataraman, L.V., Somasekaran, T and Becker, E.W. Replacement Value of Blue/green alga (*Spirulina platensis*) for Fishmeal and Vitamnin/mieral Premix for Broiler Chiks. British Poultry Science (1994) 35: 373-381

Voort, M. de, Vulsteke, E. and Visser, C. de. Macro-economics of algae. EnAlgae report WP2A7.01, in voorbereiding, 2014.

Weide, R.Y. van der, Schipperus, R., Dijk, W. van. Algae Cultivating using Digestate as Nutrient Source: Opportunities and Challenges. Proceedings European Biomass Conferention and Exhibition Hamburg, p. 1778-1784, 2014.

Vermeij, I., and J. Kanis. 2005. Bedrijfswijzer pluimvee geeft u het antwoord: Wijziging zinvol en haalbaar? De Pluimveehouderij 35:25.

11.1 Websites

- www.acrres.nl
- www.kelstein.nl
- www.algaefoodfuel.com
- www.algaecom.nl
- www.vbvoer.nl/supplementen

www.acrres.nl