Duckweed and *Azolla* as Livestock Feed for Improving Resource Efficiency and Nutritional Quality

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Abstract

Widespread soil degradation, climate change and a growing world population are all threatening global food security. Today's livestock production is coming under pressure and its role and necessity for future generations is questioned more and more.
This thesis deals with the current threats to global food security and the causes thereof. The resource consumption of global terrestrial livestock production and aquaculture are analysed in terms of resource efficiency and sustainability and discussed. Characteristics of sustainable livestock production systems are outlined and implementation strategies are provided.
The floating plants duckweed and *Azolla* and their utilization potential are described in detail as a means of providing animal feed without competing for arable land and improving the nutritional quality of animal products. Additionally, these plants can be produced in a more resource efficient manner, negating environmental pollution through fertilizer and pesticides.
The cultivation of *Azolla* is possible without any source of reactive nitrogen due its capacity to fix nitrogen from the air using cyanobacteria at rates surpassing all other known nitrogen fixing plant-bacteria symbiotic associations. *Azolla*-based agricultural systems are therefore capable of functioning without any external input of reactive nitrogen sources.
Duckweed on the other hand can consume large amounts of reactive nitrogen and produce a protein-rich biomass many times exceeding the productivity of conventional terrestrial crops. Duckweed culture has been shown to produce 20 times more protein per area and time than conventional soybean production.
On a global scale, floating plant-based systems enable a vastly improved resource efficiency in livestock production through higher yields per area and minimized environmental pollution. This thesis outlines conceptual systems that can be integrated in existing agricultural systems to decrease nutrient losses, land use area and global soil degradation. Nutrient losses on crop fields such as nitrate leaching and ammonia volatilization can be overcome to a great extent by employing pond-based systems to grow floating plants as alternative protein feed.
Feeding trials were conducted with ducks and chickens that were both fed duckweed and *Azolla* as part of their diet during a total of six trials. The effect of the floating plants in the diet on the laying performance of the poultry and the fatty acid composition of the respective eggs were investigated.
Both floating plants were found to be suitable as a partial replacement of the commercial diet of poultry. Duckweed was clearly preferred over *Azolla* by the animals, but the inclusion of both plants led to improvements in the fatty acid profile of the eggs. The effects on laying performance and feed efficiency gave mixed results.
An additional trial was also conducted with grass carp being fed with duckweed alone and their growth performance was recorded. Both floating plants were grown in ponds in Northern Germany and their biomass yield and nutritional composition was determined. The productivity of duckweed was only modest, but *Azolla* gave very high yields, compared to values from the literature.
A case study for a duckweed-based tilapia aquaculture was prepared as an example of sustainable production of animal protein that can be operated by using local inputs only and generate very high yields. The proposed system is capable of producing many times more animal protein per surface area than conventional terrestrial livestock production systems. This can be achieved without any external feed input such as fish meal and with very low pollution potential due to continuous internal nutrient recycling.

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Abbreviation and Symbols

ALA	Alpha-Linolenic Acid
B	Boron
Ca	Calcium
Cl	Chlorine
Co	Cobalt
Cr	Chromium
Cu	Copper
DHA	Docosahexaenoic Acid
DM	Dry matter
EPA	Eicosapentaenoic Acid
FA	Fatty Acid
FAO	Food and Agriculture Organization of the United Nations
FCR	Feed conversion ratio
Fe	Iron
FW	Fresh weight
gt	Giga tons = 10^6 tons
I	Iodine
K	Potassium
Mg	Magnesium
Mn	Manganese
Mo	Molybdenum
N	Nitrogen
Na	Sodium
NDF	Neutral Detergent Fiber
NH_4^+-N	Ammonium-nitrogen
Ni	Nickel
NO_3^--N	Nitrate-nitrogen
P	Phosphorus
S	Sulfur
Se	Selenium
SGR	Specific growth rate
t DM/ha/y	Yield of dry matter per hectare per year
Zn	Zinc
ω-6/ω-3 ratio	Omega-6 to Omega-3 fatty acid ratio

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1. Introduction

The world population of 7.8 billion today (2020) is expected to rise to 9.8 billion by 2050 [United Nations, 2017]. Along with the population growth, the standard of living and demand for animal products is rising.
Between 1950 and 1990, one third of global fertile soil has been lost [Millennium Ecosystem Assessment Panel, 2005]. With a rising population and declining farmland resources, severe conflicts are inevitable, if conventional farming practices will not be drastically changed. A decrease in consumption of animal products is often advised to reduce pressure on natural resources, while increased consumption of grains and legumes is advised [Willett et al., 2019].
However, evidence will be presented that not livestock production by itself, but vastly inefficient management of livestock-related resources are to blame for land degradation, pollution and related food security issues. This thesis deals with possible solution scenarios in the form of floating plant based systems, providing high quality feed for aquaculture and livestock that display exceptionally high productivity in terms of protein production, as well as superior resource efficiency.
The aim of this work is to discuss and evaluate an alternative system or system components that decrease the competition for farmland and increase the nutritional value of the animal products. The cultivation of floating plants enables the production of animal feed that is high in protein, has a very favourable ω-6/ω-3 ratio (Omega-6 to Omega-3 fatty acid ratio), while greatly exceeding productivity rates of conventional feed crops.
In this thesis, the current global use of farmland, food production and associated problems will be illustrated and compared to the proposed floating plant-based systems.
Feeding trials with ducks, chickens and fish fed with floating plants were conducted in order to assess the effects on livestock performance parameters, savings in conventional feed and improvement of nutritional characteristics in the derived animal products.
A case study was also conducted in order to evaluate the feasibility of floating plant-based aquaculture for the production of animal protein with minimal environmental impact and maximized productivity and resource efficiency.

2. Global Food Security and Challenges in the Future

The global food security is depending on a multitude of factors that are more or less connected to each other. Global population is currently at 7.8 billion and is expected to be at 9.8 billion in 2050 and 11.2 billion in 2100 [United Nations, 2017]. The global consumption of calories, proteins and animal products are steadily rising, creating additional pressure on top of a growing population.
The 17 Sustainable Development Goals established in 2015 for the year 2030 by the United Nations General Assembly include zero hunger. From 2015 to 2017, world hunger was steadily increasing, with an estimated 821 million people being undernourished in 2017 [United Nations General Assembly, 2015].
The four global main crops maize, rice, wheat and soybeans are currently providing about two thirds of the global agricultural calories. With the expected population increase, changing dietary demands and increasing biofuel production, global cereal production would have to double until 2050 according to Ray et al. (2013). The authors concluded that the current trends in yield increases are far below the suggested increases for food security in 2050.
Providing food for a growing world population with the current agricultural system is putting several planetary sub-systems under severe pressure that are also necessary for the sustained supply of the population. These sub-systems include several geo-chemical processes on a global scale that, when pushed into a certain direction too far, outside of the planetary boundaries, make sustained human habitation on this planet very unlikely [Rockström et al., 2009].
According to Liu et al. (2015) projected agricultural future scenarios often base their estimates on yield increases from intensification of water, pesticide and fertilizer input without taking into account consequential land degradation and other negative environmental impacts. However, in that case there exists a trade-off between agricultural intensification to increase yields and environmental protection. In order to secure future food security, the focus must be set on increasing the efficiency of water, fertilizer and pesticide use including the reduction of environmental impacts. Otherwise, ecological integrity will suffer damages that will translate in less yields overall, nullifying any long term intensification efforts through land degradation, loss of ecosystem services etc.
The most important processes that are also highly dependant on agricultural land use systems are outlined in the following chapters.

2.1 Soil Degradation

Soil degradation plays a major role for food security as there is no substitute for fertile land. The big majority of food production is based on soil. The Sustainable Development Goal 15.3 states: "By 2030, combat desertification, restore degraded land and soil,

including land affected by desertification, drought and floods, and strive to achieve a land degradation-neutral world." [United Nations General Assembly, 2015].
According to estimations by the FAO (2015), 33 % of all soil resources are moderately to highly degraded through erosion, compaction, salinisation, acidification, chemical pollution or nutrient depletion [Pennock et al., 2015].
The United Nations proclaimed: "From 2000 to 2015, more than one fifth of the Earth's total land area was degraded, largely due to human-induced processes, such as desertification, cropland expansion and urbanization. During the same period, there were significant productivity declines in land cover, with grasslands incurring some of the greatest losses." [United Nations General Assembly, 2015].
The Millennium Ecosystem Report indicates that between 1950 and 1990, one third of global fertile soil has been lost [Millennium Ecosystem Assessment Panel, 2005].
The FAO states: "While there is cause for optimism in some regions, the overwhelming conclusion from the regional assessments is that the majority of the world's soil resources are in only fair, poor or very poor condition." The biggest threats for global soil resources are soil erosion, loss of soil organic matter and nutrient imbalance. Global soil management challenges arise out of the nature of soils in combination with its management history.

The most important practices for sustainable soil management are given in three points:"

1. enhanced plant nutrition through balanced measures that include crop rotations with N-fixing crops, judicious use of organic and inorganic fertilizers, and targeted amendments such as lime to address specific soil chemical conditions such as high acidity,

2. minimize soil disturbance by avoiding mechanical tillage through adoption of conservation tillage and no-till systems, and

3. enhance and maintain a protective organic cover on the soil surface using cover crops and crop residues." [Pennock et al., 2015].

As later shown in section 3.1, the great majority of calories is coming from grains, typically grown in monocultures. Conventional production of seeds from annual crops are without a doubt the most soil damaging, as they have the highest dependency on mechanical tillage, due to being annuals and leave the soil bare for extended periods. The fact that more demand is placed on high yielding cropping systems due to population growth stands in stark contrast to soil degradation caused by these systems, increasing the demand even more than population growth alone.

The global soil losses from the last few decades of industrial agriculture have been nothing short of catastrophic and create the need for changed soil management systems and food production on a global scale. A safe planetary boundary limit for global land area to cropland conversion was estimated at 15 % with the current level at 11.7 % [Rockström et al., 2009]. The remaining potential land area suitable for arable land globally is estimated at 1.4 billion ha, excluding forests, built-up areas and protected areas. The current (2017) arable land coverage amounts to 1.4 billion ha according to FAO data [Alexandratos and Bruinsma, 2012].

2.2 Nitrogen Dynamics

Nitrogen is crucial for crop productivity. In pre-industrial agriculture, farmers had to rely on biological (=symbiotic) nitrogen fixation in order to supply their fields with sufficient reactive nitrogen to guarantee sustained soil fertility. This was achieved by growing symbiotic nitrogen fixing plants, mostly legumes, like clover, alfalfa and beans in rotation with other crops. It was known that these plants were crucial for long-term soil fertility long before biological nitrogen fixation was discovered in 1901.
Just seven years after that in 1908, the Haber-Bosch process was patented, the "synthesis of ammonia from its elements". It is the process that is used to manufacture reactive nitrogen from elemental nitrogen out of the atmosphere together with hydrogen gas under certain reaction conditions and the use of catalysts. This reactive nitrogen, ammonia, is further processed into ammonium, nitrate or urea based mineral fertilizers.
The Haber-Bosch process transformed agriculture like nothing else did, see figure 2.1. The amount of people that could be fed from one hectare of land increased from 1.9 persons in 1908 to 4.3 persons in 2008 according to estimates. Around 80 % of the ammonia produced in the Haber-Bosch process is used for the manufacturing of mineral fertilizers [Erisman et al., 2008].
While the usage of synthetic nitrogen fertilizers has led to substantially increased yields in a short period, it has also caused serious groundwater and air pollution.
Total global biological nitrogen fixation is estimated at 90 - 130 gt N/y (million tons of nitrogen per year). Anthropogenic activities are adding 20 gt N/y by the combustion of carbon based fuels, 40 gt N/y by the cultivation of nitrogen fixing crops such as legumes and rice and 80 gt N/y by fertilizer production (estimates from 1995). Synthetic reactive nitrogen production has surpassed biological nitrogen fixation on a global level, just some decades after its invention. Mineral fertilizer production is still rising as demand increases with increasing population [Galloway et al., 1995]. Global ammonia production in 2018 was 140 gt N [Apodaca, 2019].
Human nitrogen excretion through urine and faeces amounts to 12.5 g per person per day. For a world population of 7.7 billion this is 35 gt N/y. About 90 % of sewage is treated globally through different processes. In treatment plants the nitrogen content of the sewage is decreased, usually by nitrification and denitrification processes, before it gets discharged into the waterways. Recovery and reuse of the nitrogen out of sewage is possible, but still not commonly practised [Ronteltap and Sirait, 2010].
In order to illustrate global nitrogen use efficiency in food production, the nitrogen contained in human excreta in comparison to applied mineral nitrogen fertilizer and biologically fixed nitrogen in agricultural systems can be estimated:
Global reactive nitrogen synthesis in 2018 was 140 gt N. Of that, 88 % were used for the production of fertilizer, which equals 123.2 gt N/y [Apodaca, 2019].
Biological nitrogen fixation estimates for pastures and fodder legume production range from 12 to 25 gt N/y [Mia et al., 2018]. Estimates for biological nitrogen fixation of global crop legumes vary strongly with values between 25 and 70 gt N/y [Adams et al., 2018].
Adding up the average of both cropland (47.5 gt N/y) and pastures and fodder legumes (13.5 gt N/y) accounts for the total biological nitrogen fixation of global agriculture (61 gt N/y). Combined with the most current data (2018) for applied mineral nitrogen fertilizer (123.2 gt N/y) amounts to a total reactive nitrogen input/fixation of 184.2 gt N/y on agricultural lands. The nitrogen in human excreta of 7.63 billion (for 2018) is at 34.8 gt N/y, which is 19 % of total reactive nitrogen input/fixation. Consequently, 81 % of the nitrogen are lost, before being consumed by humans in the form of animal and

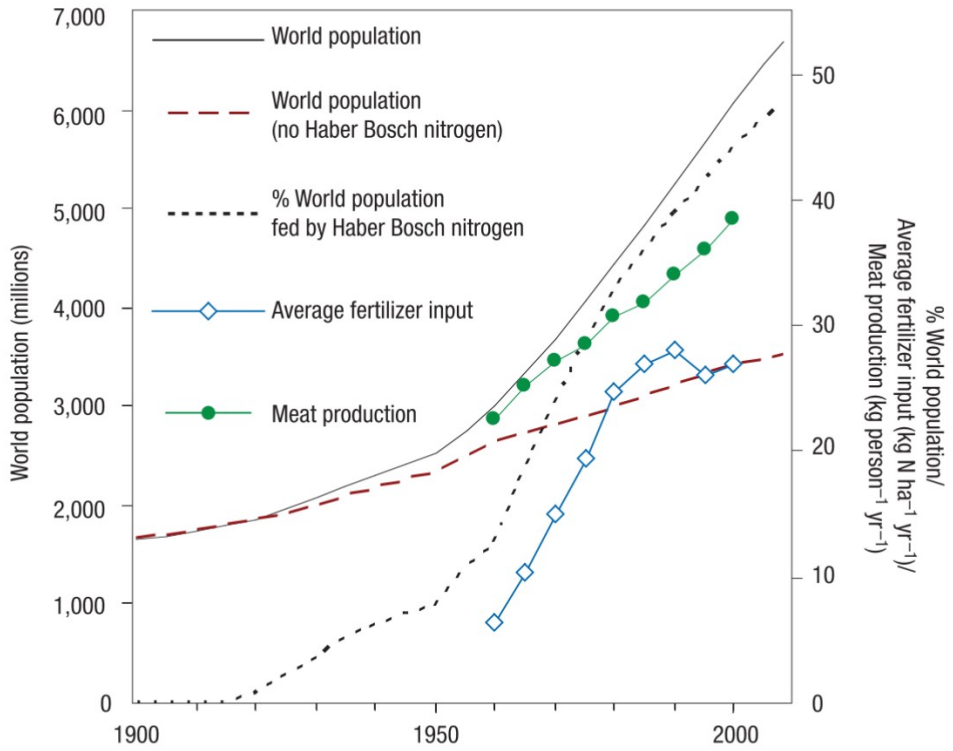

Figure 2.1: Trends in human population and nitrogen use throughout the twentieth century. Of the total world population (solid line), an estimate is made of the number of people that could be sustained without reactive nitrogen from the Haber–Bosch process (long dashed line), also expressed as a percentage of the global population (short dashed line). The recorded increase in average fertilizer use per hectare of agricultural land (blue symbols) and the increase in per capita meat production (green symbols) is also shown, after Erisman et al. (2008).

vegetal protein.
Nitrogen application rates in agriculture are rising as a consequence of decreasing nitrogen efficiency, which describes the amount of nitrogen retrieved in the produce relative to the applied amount. Global nitrogen efficiency in cereal production declined from 80 % in 1960 to just 30 % in 2000. In 2005, of 100 gt of nitrogen that were produced, only 17 gt were consumed by people in the form of crops, dairy and meat products. Decreasing nitrogen efficiency and an increasing population are both raising the demand for synthetic nitrogen.
Ongoing loss of soil fertility is among the reasons for the extremely low efficiency of mineral nitrogen fertilizer [Erisman et al., 2008]. The nitrogen use efficiency is strongly depending on the ratio between mineral nitrogen fertilizer and biological nitrogen fixation on global cropland. The more mineral nitrogen fertilizer is used in relation to biological nitrogen fixation, the lower the nitrogen use efficiency [Lassaletta et al., 2014].

The global nitrogen flow entering the ocean via river discharge was estimated at 36.7 gt/y in 1970 and 43.2 gt/y in 2000 [Seitzinger et al., 2010]. A more recent value for reactive nitrogen riverflow was estimated at 62 gt N/y. Moreover, groundwater inflow was estimated at 15 gt N/y, biospheric increment at 9 gt N/y, atmospheric transport to the ocean at 54 gt N/y and denitrification of reactive nitrogen at 100 gt N/y. However,

the author stated that there was a discrepancy between reactive nitrogen input and fate, as the fate of 46 gt N/y was not accounted for [Schlesinger, 2009].

The production of mineral nitrogen fertilizer constitutes the highest energy demand of conventional crop production, with 36 % of total energy demand, mostly supplied by fossil fuels [Bardi et al., 2013]. The production of mineral nitrogen fertilizer is causing widespread pollution of water bodies, negatively impacting water quality, land value and species diversity. Oxygen declines in estuaries and coastal systems are being caused by an excess of nutrients, mainly nitrogen and phosphorus in the watershed from agriculture and sewage. Oxygen loss is one of the most important changes of oceans caused by human activity and can cause major changes in ocean productivity, biodiversity and biogeochemical cycles [Breitburg et al., 2018].

One extreme example of the natural capital impacts arising from mineral fertilizer is the conventional wheat production in Germany. Water pollution from nitrate leaching was found to account for 95 % of total natural capital impacts, including air pollution, soil pollution, greenhouse gases and land use change. Estimates suggested a natural capital cost from water pollution of 2,396 US$ for 1 ton of wheat produced in Germany. This is more than 10 times the market value of 1 ton of wheat. The production of organic wheat reduced the water pollution by nitrates down to 1,274 US$ per produced ton, still considerably higher than the market value of organic wheat [Food and Agriculture Organization of the United Nations, 2015].
In the EU for the year 2008, the economic benefit of nitrogen fertilizers in primary agricultural production was estimated at 20 - 80 billion €/y, while the cost of pollution ranged between 35 - 230 billion €/y. Total social costs (including pollution) were estimated at 75 - 485 billion €/y [van Grinsven et al., 2013].
Of the reactive nitrogen lost during the production of goods and services in the US, 66 % are estimated to enter the air in the form of nitrogen oxides, ammonia, nitrous oxide and elemental nitrogen contributing to smog formation, acid rain, eutrophication, loss of biodiversity and climate change. The remaining 34 % are lost to waterways resulting in water pollution and anoxic zones by algae blooms [Houlton et al., 2013].
According to estimates, about 25 % of nitrogen applied as fertilizer in the US is lost to the atmosphere in the form of ammonia. Ammonia is a major contributor to the formation of particulate matter with a diameter of less than 2.5 µm (PM2.5) by combining with nitrates or sulphates. Breathing in of these particles constitutes a well-documented human health concern, negatively affecting the respiratory system, and a major premature mortality factor. The resulting health costs from PM2.5 from food export from the US is estimated at 36 billion US$ for 2006, or 100 US$ per kg of ammonia. The net value of these exports for 2006 was 23.5 billion US$. In essence, one single aspect out of many other negative impacts resulting out of mineral nitrogen fertilizer use already surpasses the net value of the agricultural products in the form of increased health costs (morbidity and mortality) as a consequence [Paulot and Jacob, 2014].
The planetary boundary of reactive nitrogen synthesis from atmospheric nitrogen is estimated at 35 gt N/y as an acceptable limit that ensures a safe operating space for humanity on this planet. Current production rates have already surpassed this limit four-fold [Rockström et al., 2009].
To summarize, global nitrogen requirements are rising due to decreasing nitrogen fertilizer efficiency. Resulting costs of pollution and health costs are surpassing the economic benefits derived from nitrogen fertilization.

2.3 Peak Phosphorus

Agriculture is the main user of phosphorus, using 80 - 90 % of global supplies. Phosphorus, in the form of phosphates, together with nitrogen and potassium constitutes the main three fertilizer components of mineral fertilizer used in conventional agriculture. Modern food production is depending on a steady supply of phosphates that are being mined from fossilized reserves and processed into fertilizer.
These reserves are expected to last for 100 - 300 years at the current production rates. Before fossil reserves are emptied, recovery technologies have to be adapted and fertilizer efficiency has to be increased for sustained food production.
Global phosphorus fertilizer efficiency is quite low, comparable to that of nitrogen. Applied phosphorus is taken up by only 15 - 30 % by crops, while 33 % is lost by wind and water erosion. The rest stays in the soil and forms insoluble salts that are not directly available to plants [Tirado and Allsopp, 2012].
In 2017, 45.5 gt of phosphate, which is equal to 19.8 gt of phosphorus was applied in agriculture globally [Food and Agriculture Organization of the United Nations, nd].
The phosphorus in human excreta amounts to 1.5 g/d per person or 4.1 gt P/y for a world population of 7.55 billion (in 2017) [Ronteltap and Sirait, 2010]. Hence, only about 21 % of the phosphorus that is applied on the fields, is finally consumed by humans.
Unlike nitrogen, the volatilization of phosphorus is negligible. However, the combined losses of harvests, wind and water erosion and run-off on agricultural lands are occurring at a rate that is resulting in an estimated net loss of 10.5 gt P/y on the world's croplands [Liu et al., 2008b].
Around 20 gt of phosphorus are mined per year, while 8.5 - 9.5 gt/y gets lost to the ocean, contributing to eutrophication, excessive algal blooms and anoxic zones. Pre-industrial inflow of phosphorus into the ocean is estimated at 1 gt/y. Current levels are estimated to be close to planetary boundaries, set at 11 gt/y where ocean anoxic events become probable, according to modelling [Rockström et al., 2009].
According to estimates, the global phosphorus flow into the ocean was 7.6 gt in 1970 and 8.6 gt in 2000 [Seitzinger et al., 2010].
Phosphorus fertilizers are also problematic due to heavy metal contamination, as they contain cadmium, fluoride and uranium, still after processing [Tirado and Allsopp, 2012]. According to long-term mass balance modelling of European crop land, the cadmium deposition in soil, leaching into the ground water, as well as cadmium plant uptake will rise under current fertilizer utilization rates. Average external input of cadmium onto crop land soils is 21.5 g/ha/y, whereas the big majority stems from mineral phosphate fertilizers. In mineral phosphate fertilizers in California, the average concentration of cadmium was measured at 89 mg/kg [Chen et al., 2007]. Cadmium is highly toxic. A correlation between cadmium concentration in soils and water and the occurrence of breast- and prostate cancer could be found for 26 European countries. Phosphate rock fertilizer is estimated to be the single biggest source of cadmium pollution, after atmospheric deposition and sewage sludge [Pan et al., 2010]. In India, the use of single super phosphate fertilizer was correlated with the fluoride concentration in the drinking water, putting residents at risk of fluoride poisoning [Kundu and Mandal, 2009].
Besides phosphorus, there are other elements that might be depleted in the near future, probably even before the depletion of phosphorus. According to estimates, global reserves of manganese, copper, boron, molybdenum and nickel will be depleted in less than 50 years from now. For zinc, it is estimated at less than 20 years [Dimkpa and Bindraban, 2016]. All these elements are essential plant nutrients.
Assuming an average dietary intake of 10 mg of zinc per person per day, the loss of zinc

at the current global population through the sewage system is about twice as high as the global industrial demand for 2014. Hence, just like for phosphorus, suitable nutrient recovering strategies from sewage have to be employed urgently.

2.4 Climate Change

Anthropogenic climate change is generally assumed to be the consequence of elevated greenhouse gases in the atmosphere, mainly carbon dioxide stemming from the combustion of fossil fuels. Atmospheric concentrations of carbon dioxide at pre-industrial times were at 280 ppm, while they have risen to 387 ppm at present. A safe planetary boundary level has been estimated at 350 ppm. Accordingly, radiative forcing, being the difference between sunlight absorbed by the earth and energy radiated back into space is at 1.5 W/m^2 at the present, while a safe boundary limit was established at 1 W/m^2 [Rockström et al., 2009].
The effect of land use systems on radiative forcing, however, might be underestimated. A water saturated field with a dense vegetation cover is able to convert 70 - 80 % of the sunlight energy by water phase change through evapotranspiration, which has a cooling effect on the atmosphere, as shown in figure 2.2. A drained landscape might only achieve 5 - 10 %. Land owners are having a strong impact on the microclimate of their land by lowering the water table and decreasing vegetation cover by converting natural woodlands or wetlands into agricultural land. Not only the conversion of water into water vapour, but also the increased air humidity, as well as cloud formation has a regulating effect on the climate. Removal of dissipative structures results in increased temperature and air pressure potentials affecting regional climate stability including rainfall patterns [Pokorny, 2001].
In the past 300 years, on a global level, rain-fed cropland and pastureland has increased

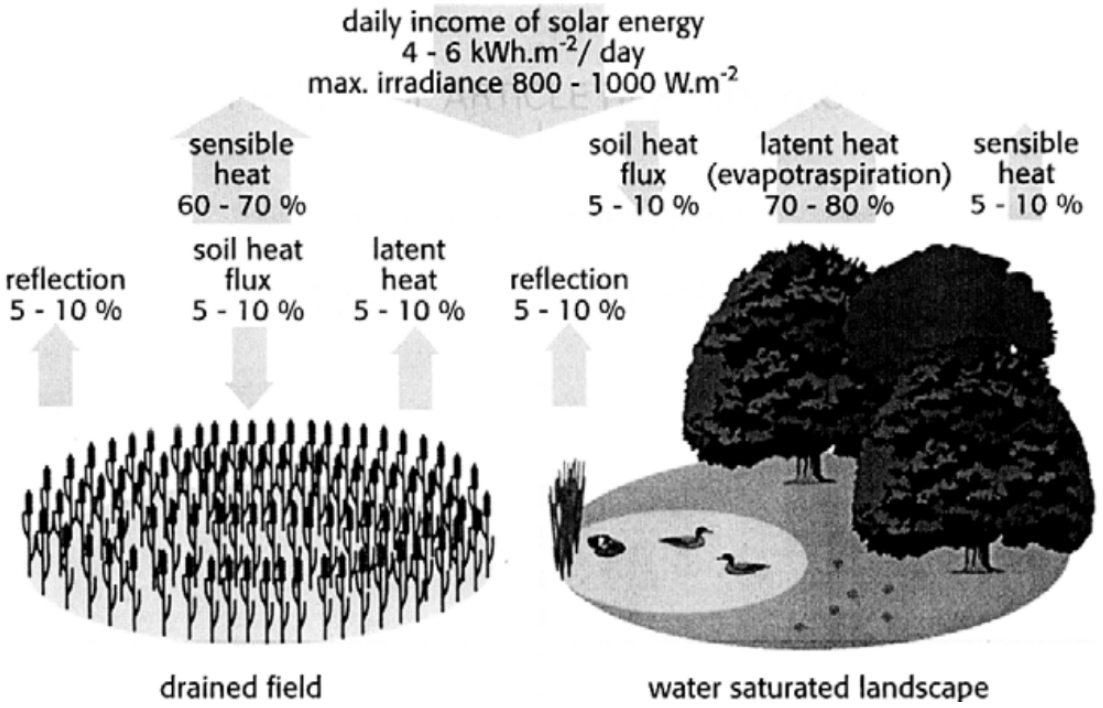

Figure 2.2: Dissipation of solar energy on 1 m^2 vegetation-covered landscape. of drained landscape and on 1 m^2 of water-saturated and , from [Pokorny, 2001].

460 % and 560 % respectively, which is decreasing evapotranspiration rates area-wide. Both recharge of ground water as well as streamflow of rivers have increased as a consequence, clearly demonstrating that the proportion of rainwater that gets evaporated on the global land area has declined [Scanlon et al., 2007].
Irregularity in rainfall patterns are expected as a consequence of climate change. By 2050, 0.5 to 3.1 billion people are expected to be exposed to increased water scarcity as a consequence of climate change [Gosling and Arnell, 2016]. At the same time, the global flood risk will increase by 187 % due to climate change. The current 100-year flood will occur at least twice as often across 40 % of global area [Arnell and Gosling, 2016].
About 10 - 12 % of global greenhouse gas emissions stem from agriculture [Chai et al., 2019]. If increasing atmospheric carbon dioxide levels or a decrease in dissipative structures inducing water phase change is the main driver for climate change is still controversial. The causes for climate change are highly complex and involve a multitude of additional factors such as sun activity, earth magnetic field changes, cloud formation and many more, all of them influencing each other to a certain extent. The IPCC (Intergovernmental Panel on Climate Change), which supports the view that atmospheric carbon dioxide is the main contributor to climate change states: "In climate research and modelling, we should recognise that we are dealing with a coupled non-linear chaotic system, and therefore that the long-term prediction of future climate states is not possible."
The fact that the land use of agriculture has a substantial effect on climate change is however out of debate. According to the IPCC: "Land-use change results in changing the physical and biological properties of the land surface and thus the climate system." [Houghton et al., 2001]

2.5 Species Diversity

The current loss of biodiversity is not slowing down. Main indicators of pressures on biodiversity such as "resource consumption, invasive alien species, nitrogen pollution, overexploitation, and climate change impacts" are still increasing [Deal et al., 2010]. The pre-industrial rate of biodiversity loss is estimated at 0.1 - 1 species per million species going extinct per year. The current rate is at over 100, while a safe operating space threshold is estimated at 10.
Land use changes are given as the main driving force behind biodiversity loss, such as conversion of natural ecosystems into agricultural land.
The accelerated extinction rate can have widespread effects that are interacting with other planetary boundaries as well. Species diversity has an impact on ecosystem resilience, which becomes especially important in the face of climate change [Rockström et al., 2009].
The main drivers for loss of biodiversity are linked to the intensification of agriculture including pesticide use. Ecosystem services such as biological pest control and pollination are directly linked to biodiversity. Pollination alone is estimated at 14 US$ per hectare of land [Butler et al., 2007]. Hallmann et al. (2017) reported a decline in flying insects of more than 75 % in Germany during the past 27 years.

2.6 Conclusion

Rockström et al. (2009) argue that "a rapidly growing reliance on fossil fuels and industrialized forms of agriculture" are the main causes for destabilizing the planet's environ-

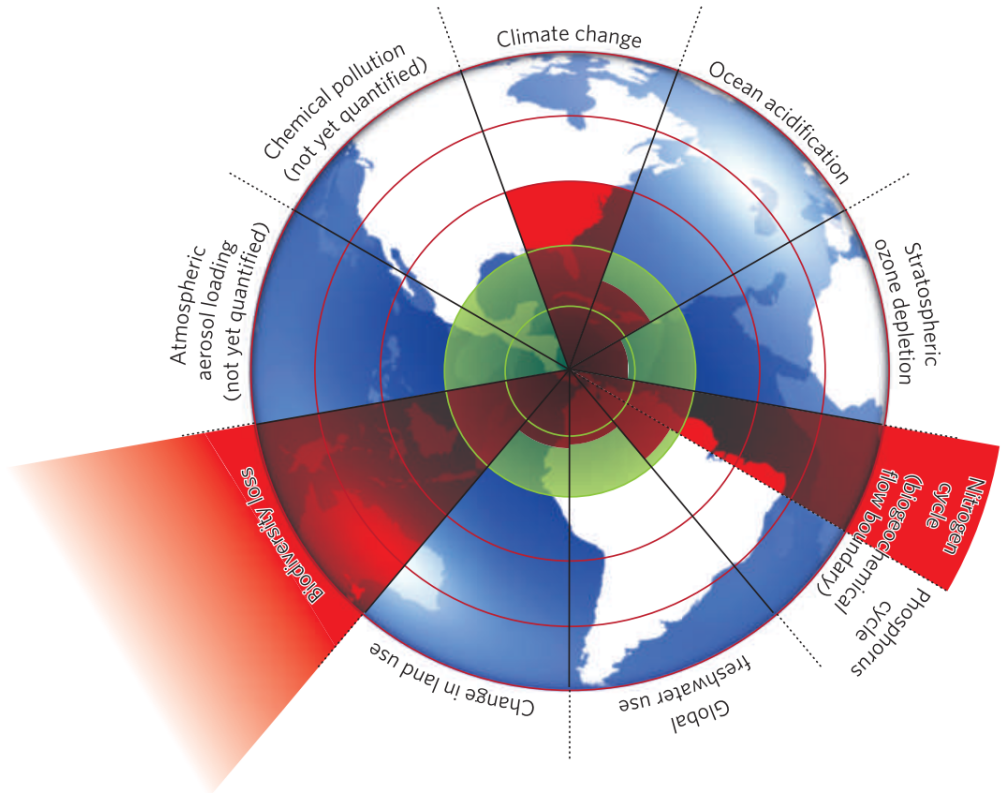

Figure 2.3: Beyond the boundary. The inner green shading represents the proposed safe operating space for nine planetary systems. The red wedges represent an estimate of the current position for each variable. The boundaries in three systems (rate of biodiversity loss, climate change and human interference with the nitrogen cycle), have already been exceeded, after [Rockström et al., 2009].

ment. Out of nine of their proposed planetary boundaries, three are already overstepped: climate change, biodiversity loss and the nitrogen cycle, as shown in figure 2.3.
Further intensification of agriculture in order to sustain food security of a growing population experiencing an accelerating rate of soil degradation will aggravate the underlying causes even more.
The next chapter will deal with the current resource consumption of global agriculture, specifically in regards to livestock production. What makes current livestock production practices a global threat for food security and how they can be transformed into sustainable systems will also be discussed.

3. Resource Consumption and Livestock Production

3.1 Current Agricultural Land Use per Capita

In the following chapter, the current (data from 2013 - 2017) global land use of agriculture is illustrated. In order to provide a more tangible understanding of the corresponding data, it is shown for one person of global average. As can be seen in figure 3.1, agricultural area presents the biggest fraction of the whole global land area with 37.4 %. After that comes forest land with 30.7 % and after that barren lands with 14.5 %. Artificial surfaces like urban areas only account for 0.4 %. Of the agricultural land, two thirds are under permanent meadows and pastures. A small fraction of 3.4 % is dedicated to permanent crops (plants that produce for more than 5 years) and on the rest, 29.3 %, temporary crops are grown (figure 3.2). The global area distribution of the temporary crops is shown in figure 3.3. Almost half of the global crop area is dedicated to wheat, maize, rice and soybeans or about 14.5 % of the total global agricultural area. Further details on agricultural land allocation can be found in the Appendix A (table A.1).

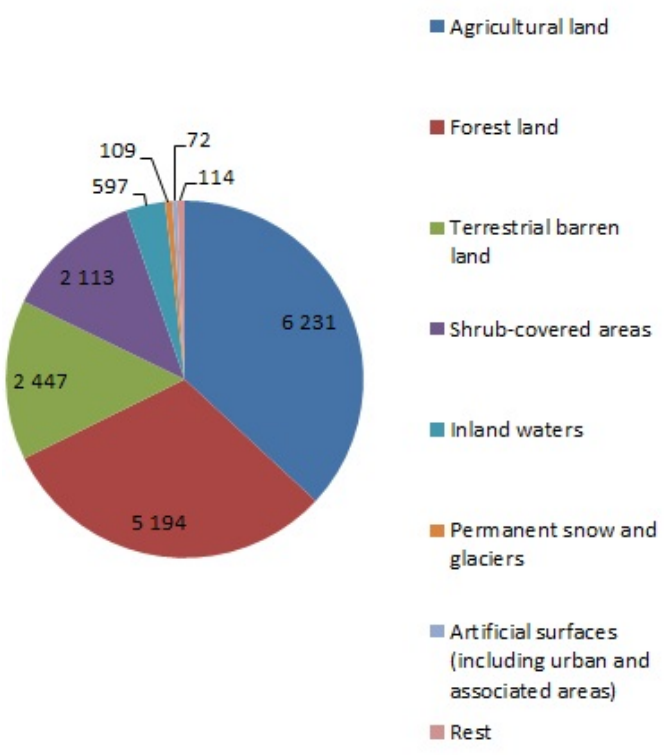

Figure 3.1: Land area composition per capita in m^2, after FAO statistics for 2015.

These four crops are supplying about two thirds of global agricultural calories [Ray et al., 2013]. In figure 3.4 the global daily calorie supply is expressed as calories from the main animal and vegetal products per capita. In figure 3.5 the same is shown for global protein supply.

On figure 3.6 and 3.7 the global average calorie and protein intake from both vegetal and animal products can be seen from 1963 to 2013. While all values are steadily rising, animal products show a more pronounced relative increase in grams of consumed protein

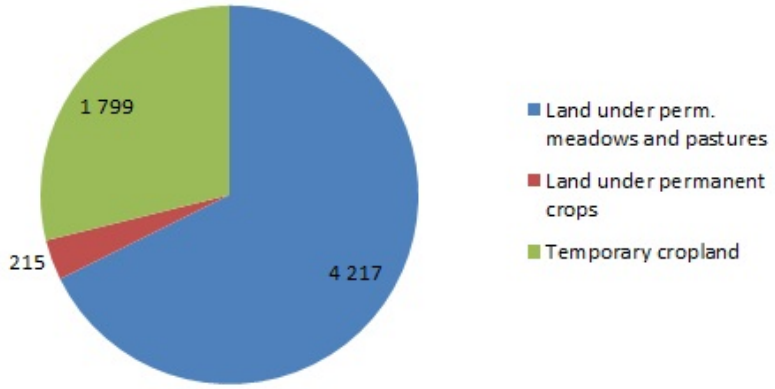

Figure 3.2: Agricultural land area composition per capita in m^2, after FAO statistics for 2015.

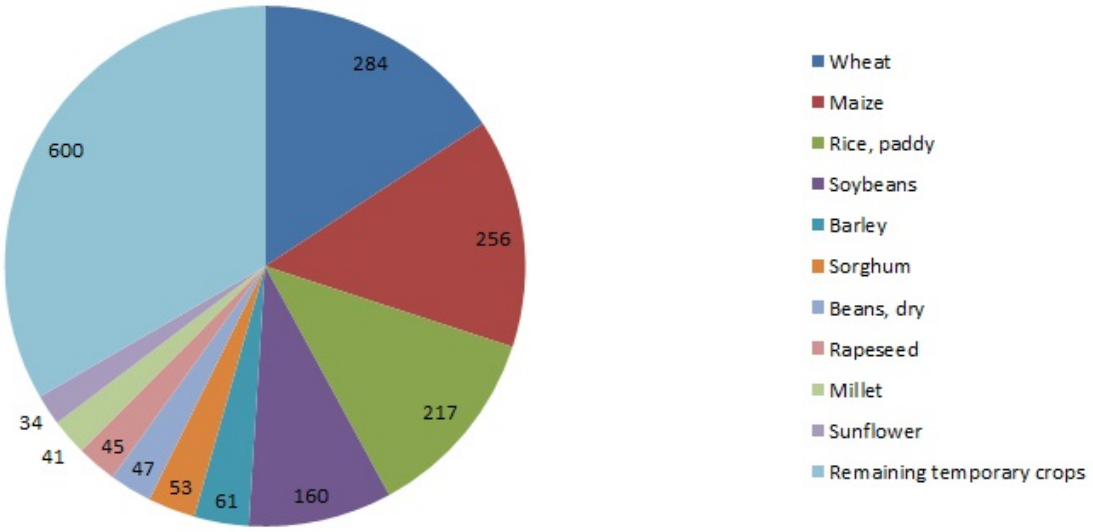

Figure 3.3: Temporary cropland composition, after FAO statistics for 2015.

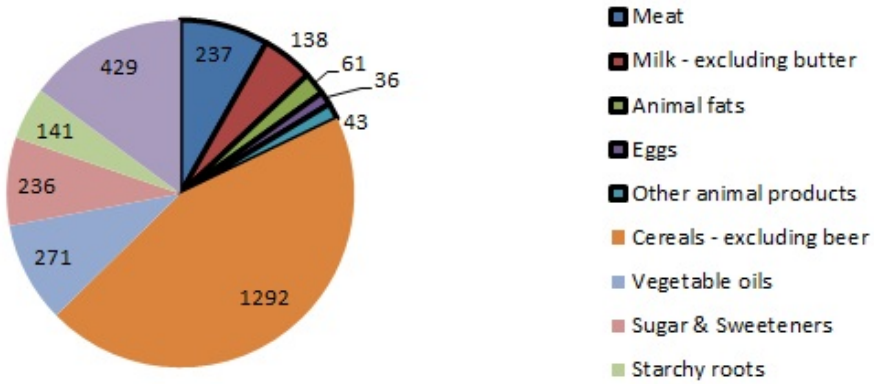

Figure 3.4: Calorie supply by food products per capita per day expressed in [kcal/capita/day], after FAO statistics for 2013.

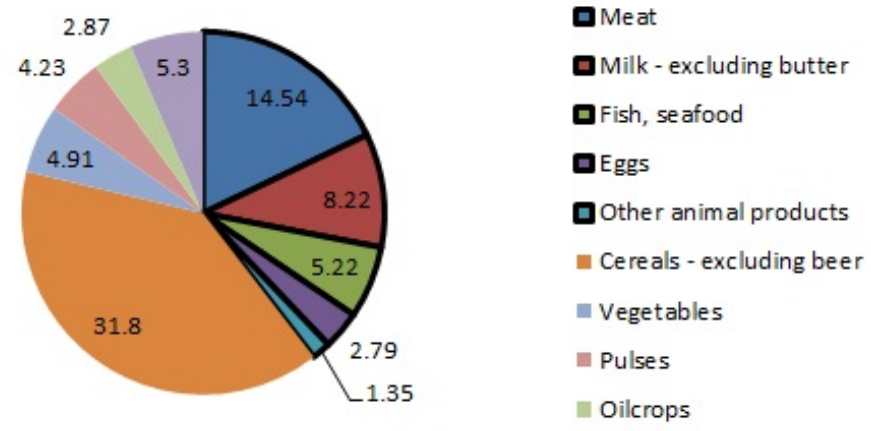

Figure 3.5: Protein supply by food products per capita per day expressed in [g/capita/day], after FAO statistic for 2013.

as well as the amount of calories than vegetal products.
At present, 16 % of human-edible crops are being used for non-food purposes. Increased demand for biofuels will likely put global food production under pressure [Berners-Lee et al., 2018].
Alexandratos et al. (2012) are estimating an increase of per capita calorie consumption of 2,772 kcal/day for 2005/2007 to 3,070 kcal/day in 2050 and an increase of meat consumption from 38.7 kg/y to 49.4 kg/y for the same time frame.

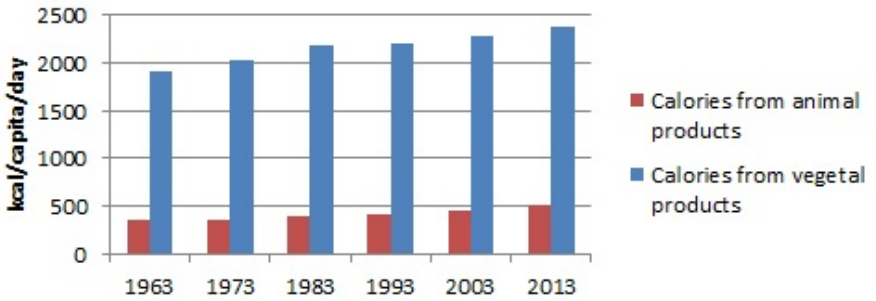

Figure 3.6: Global calorie supply per capita from vegetal and animal products from 1963 - 2013, after FAO statistics.

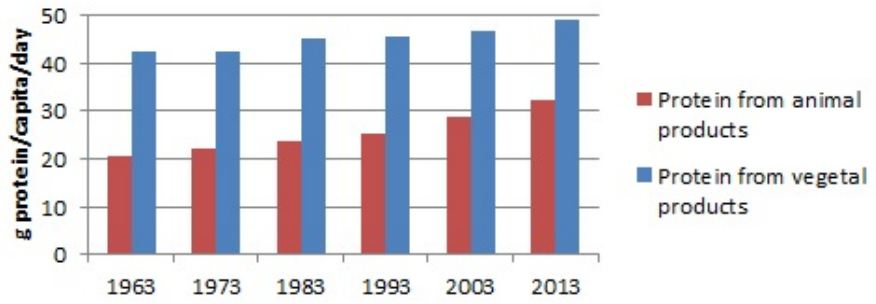

Figure 3.7: Global protein supply per capita from vegetal and animal products from 1963 - 2013, after FAO statistics.

3.2 Resource Consumption of Current Global Livestock Production

3.2.1 Terrestrial Livestock Production

About 45 % of the protein feed share in the global livestock sector comes from grass and leaves, mainly fed to ruminants and 14 % comes from oilcrops (rapeseed, sunflower, cotton etc.), 10 % from soy, 10 % from cereals, 10 % from crop residues and 9 % from by-products and other sources. The proportion of feed production area of cropland has increased from 1970 to 1983 peaking at 46 % and 51 % respectively. Due to substantial yield increases by a factor of 2.7 for global cropland, this proportion decreased to about 37 % nowadays, even though feed production quantity increased tremendously.
Berners-Lee et al. (2018) estimated that 34 % human-edible crop calories are being fed to livestock.
In the last decades, livestock production has undergone dramatic changes, as illustrated in figure 3.8. Between 1961 and 2013, the pasture land area increased by only 5 %, while the crop area increased by 13 %. All livestock production quantities increased tremendously due to population increase and increased demand for animal products. However, there has been a substantial change in the ratio of herbivore to omnivore derived products. The increase in omnivore products from monogastric animals including pigs, poultry and fish was much higher than the increase in herbivore animal products from polygastrics or ruminants including cattle, mutton (sheep) and goats. The latter are primarily adapted to rely on grass, herbs and leaves, while monogastrics require more energy dense feed and naturally feed on animal protein as well.
Research on intensive livestock production has doubled the efficiency of pigs and chicken converting grain into meat in the last 30 years. As a consequence, prices for meat have been dropping, while cereals for human consumption have increased in prices [Tester and Langridge, 2010].

3.2.2 Aquaculture

Aquaculture is the fastest growing food production industry since 1980, with production quantities growing faster than the global population. In 2014 for the first time ever, more fish for human consumption was produced in aquaculture than was caught in fisheries. For 2030 aquaculture is expected to provide 60 % of all fish for human consumption [FAO, 2018].
Aquaculture relies on fishmeal as part of the diet of fish and crustaceans. Of the global fishmeal production, 2 % were used in aquaculture in 1960, 10 % in 1980 and 73 % in 2010, while pigs received 20 % and chicken 5 % in the same year [Shepherd and Jackson, 2013]. Of the world fishery stocks, 30 % are overfished, 60 % are fully fished and less than 10 % have remaining capacity [Little et al., 2016]. Global average fish consumption per capita grew from 9.0 kg in 1961 to 20.2 kg in 2015 [FAO, 2018].
The percentage of fishmeal in aquaculture feed formulations lies between 0 to 50 %, while the percentage of fish oil is between 0 - 25 %. Species like molluscs or filter feeding carps can be farmed without wild caught fish, while farmed marine finfish, eel, salmon and trout require a high input of wild fish in their feed [Naylor et al., 2000]. In 2016, aquaculture produced 80.0 gt of fish, while 90.9 gt were caught in fisheries, of which 19.7 gt were used as feed ingredient [FAO, 2018]. Most fishmeal in aquaculture is used for crustaceans (29 %), followed by salmonids (24 %) and marine fishes (23 %) [Shepherd and Jackson, 2013].
While aquaculture production is rapidly expanding, global wild catches are stagnating

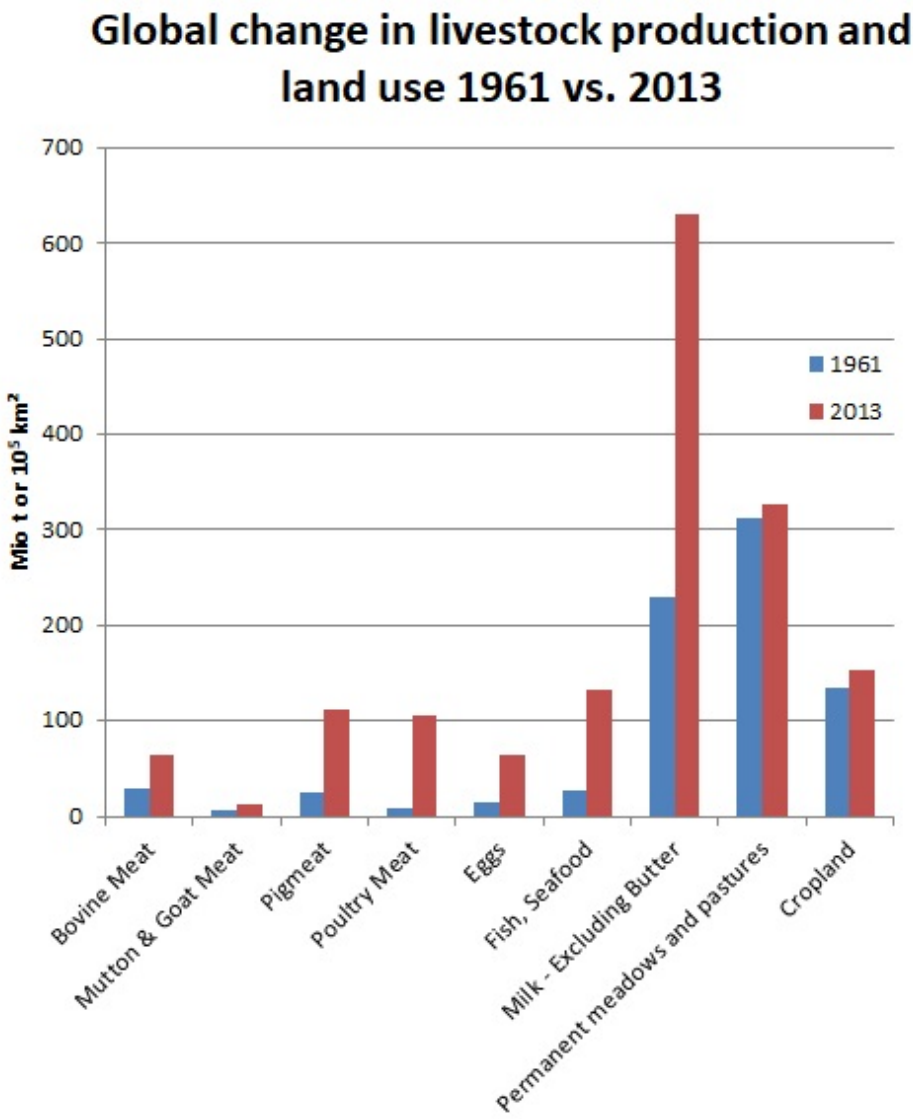

Figure 3.8: Global change in livestock production and agricultural land use in 1961 vs. 2013, after FAO statistics.

and are also expected to remain near current levels. Forage fish, mainly anchovies, herring and sardines are required as a source of micronutrients, especially long-chain ω-3 fatty acids (FAs) EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid). Concerns over sustainability issues involving the use of fishmeal and fish oil in aquaculture are increasing as ecological limits of forage fish production are becoming apparent.
However, only in the 2000s did aquaculture become the main consumer of forage fish. Before that they were mainly used for pig and poultry production. Through the rapid growth of aquaculture, the use of fishmeal for pigs and poultry has declined, while demand for fishmeal and fish oil alternatives for aquaculture are rising. Some alternative inputs include algae, insects, yeasts and bacteria [Froehlich et al., 2018]. Over the last decades the percentage of fishmeal and fish oil in aquaculture feed formulations has steadily declined, while it has been replaced with alternative proteins and starch and vegetable oils, mainly from soybeans, other oil crops and grains [Shepherd and Jackson, 2013].

3.2.3 Land Use of Animal Protein Production

While livestock occupies the permanent meadows and pastures that make up about two thirds of the global agricultural area, there is also about 35 - 39 % of the temporary cropping area dedicated to livestock fodder production, according to estimates [Manceron et al., 2014]. Precise data on the total land share for fodder production is not available, as many crops that are used as fodder, are grown for multiple products. For example oil-cakes that are fed to livestock are a by-product of vegetable oil production, for human consumption as well as for biodiesel production. Another example would be bran, which is also used as fodder, a by-product of grain processing [Manceron et al., 2014].
Therefore, the total area percentage of agricultural land used for livestock lies at around 78.8 %, composed of 67.3 % of permanent meadows and pastures and about 37 % of the residual crop land (32.7 %) for feed production. The global area of land dedicated to livestock production is almost the same area as of global forests. However, concerning the global diet, animal products account for 39.6 % of dietary protein and only for 17.8 % of dietary calories (figure 3.5 and 3.4).

The global amount of animal protein produced per year is at around 91 gt (calculation attached in the Appendix A, table A.2), consisting of meat, milk, fish, seafood, eggs, offals, other aquatic products and animal fats. Hence, the global animal protein productivity equals around 23.8 kg/ha/y. This figure also includes fish and seafood from the ocean, lakes and rivers, not included in the agricultural area.
The current protein productivity in kg protein/ha/y for different agricultural products can be seen in two different data sets in figure 3.9 [Clark and Tilman, 2017] and figure 3.10 [Poore and Nemecek, 2018]. In general, plant protein crops are more area-efficient, with legumes at the top, especially soybeans. Herbivore systems generally have the lowest protein production per area [Poore and Nemecek, 2018]. In aquaculture, the

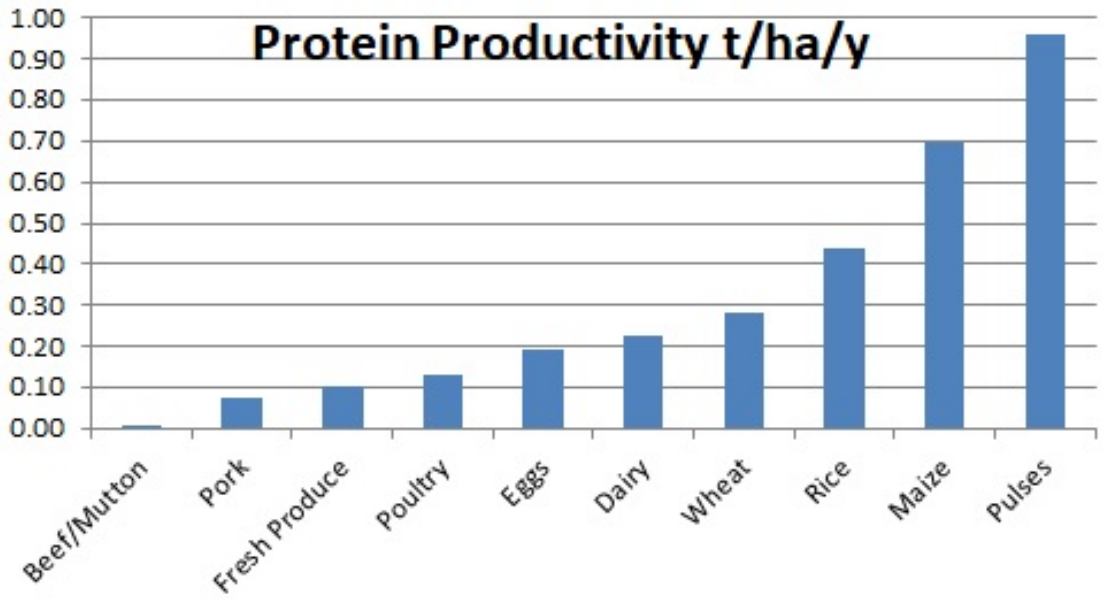

Figure 3.9: Average protein productivity of some livestock and crop production systems, after Clark and Tilman (2017).

farming of most fish and crustaceans requires a certain proportion of fishmeal in the feed formulations, which has no associated land use, as they are produced on land area. Hence, in these graphics, aquaculture operations look much more efficient than they

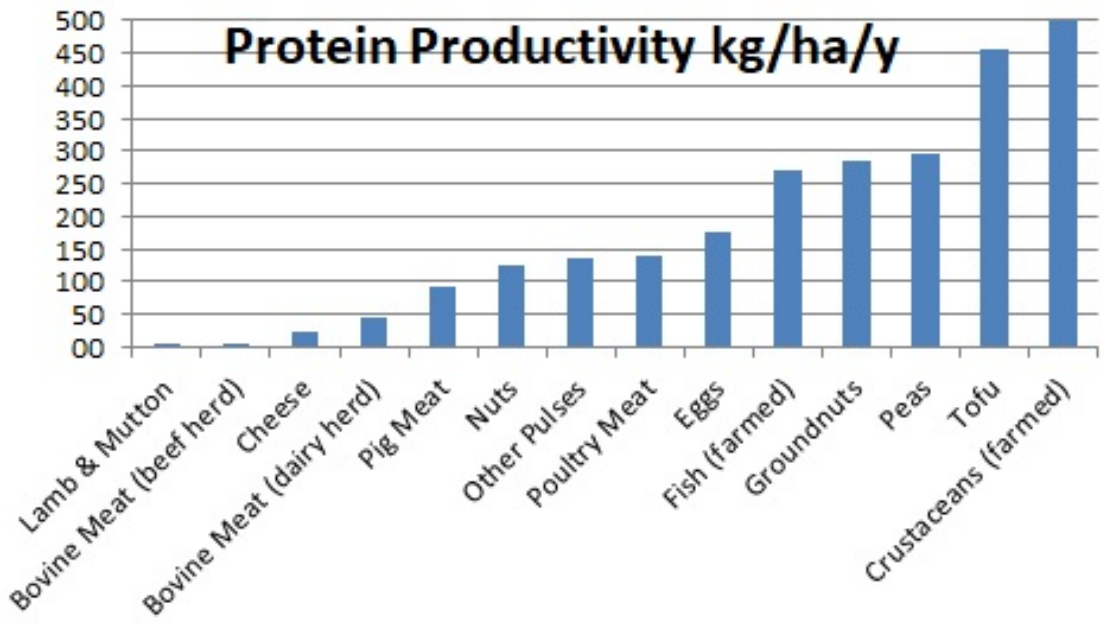

Figure 3.10: Average protein productivity of some livestock and crop production systems, after Poore and Nemecek (2018).

truly are, when just land use is considered. The same effect has to be considered for pigs and poultry, as they also receive fishmeal in their diet, but to a lesser extent [Shepherd and Jackson, 2013].

3.3 Food Production in the Context of Sustainable Planetary Boundaries

The EAT-Lancet Commission outlined a reference diet in a paper from 2019, which is based on both planetary boundaries and dietary recommendations on a global level. As planetary boundaries they are taking into account climate change, nitrogen cycling, phosphorus cycling, freshwater use, biodiversity loss and land-system change. Their proposed diet in relation to the current global diet can be seen in figure 3.11. They suggest substantial reductions for red meat and starchy vegetables and increased intake of dairy, fruit, legumes, whole grains and nuts. It is argued that the way the global population has been eating for the past 50 years is the main driver of climate change and biodiversity loss [Willett et al., 2019].

Berners-Lee et al. (2018) argues that current agricultural production is sufficient to feed the world population in 2050, however only if animal product consumption is drastically restricted and less human-edible crops are fed to livestock and are directly used for human consumption. Considering a business as usual scenario, crop yields would have to be increased by 119 % until 2050 to feed an expected population of 9.7 billion. Tilman et al. (2015) states that 60 % of the global grain production is directly consumed, while 35 % is used as animal feed and 5 % for biofuels. In wealthy countries, 8,000 kcal of crops are necessary to produce a typical diet containing 3,500 kcal per day, as most calories are needed as animal feed. A population increase of 30 % in 2050 would require a 100 % production increase in agriculture, as demand for animal products is expected to greatly increase, especially in developing countries. Adopting a Mediterranean

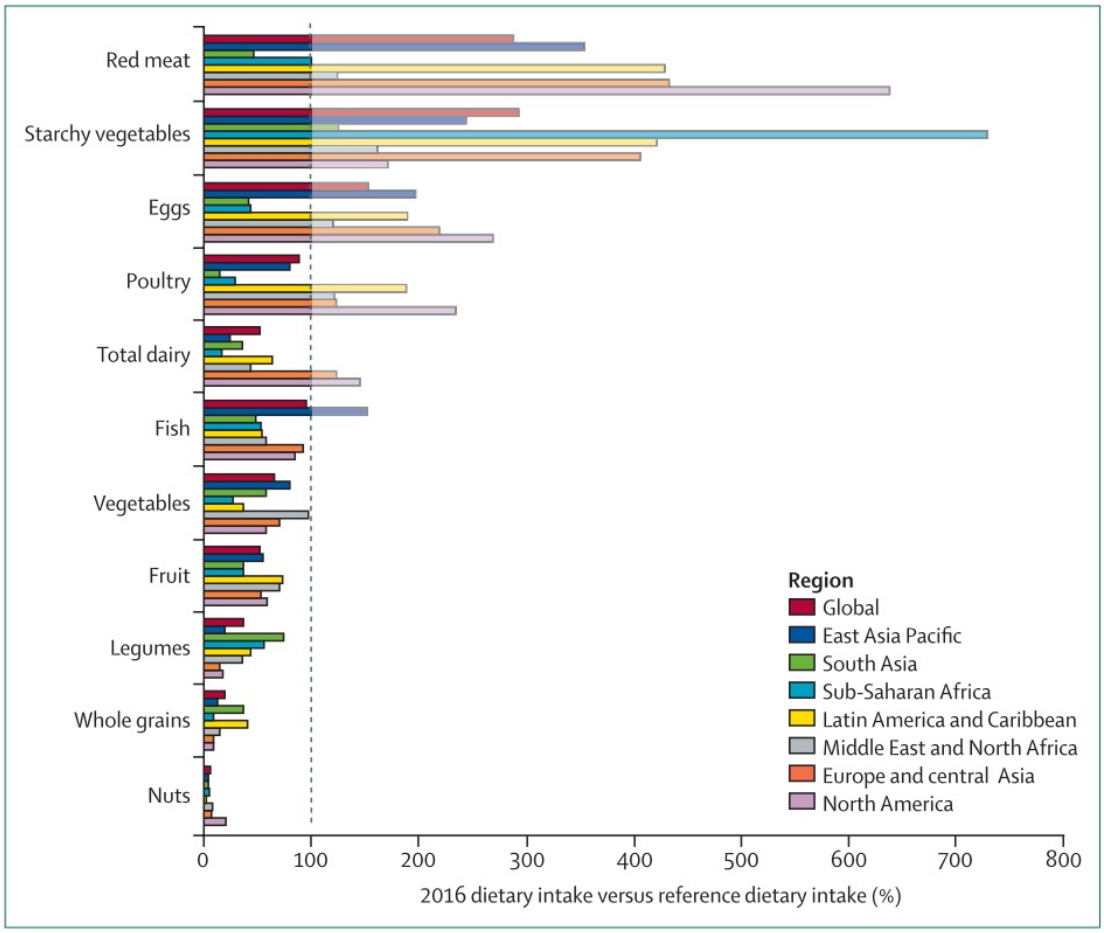

Figure 3.11: Diet gap between dietary patterns in 2016 and reference diet intakes of food. The dotted line represents intakes in reference diet, after Willett et al. (2019).

or vegetarian diet is proposed as a means of reducing environmental burden in future agricultural scenarios, due to lower animal product consumption.
Alexandratos et al. (2012) estimate the global production increase for total agricultural production and meat production from 2005/2007 to 2050 at 60 % and 76 %, respectively. These production increases over this time period are projected to be by 90 % the result of agricultural intensification.
In order to achieve the needed doubling of agricultural production in 2050, 170 % increases in nitrogen fertilizer, 140 % increase in phosphate fertilizer, 190 % increase in irrigation and 170 % increase in pesticide usage is expected to be necessary for intensification purposes. Only 23 % increase in cropland and 16 % increase in pastures are predicted, as the main driver for production increases is clearly seen in intensification [Tilman and Clark, 2015].
A review on the sustainability of the omnivorous diet, vegetarian diet and vegan diet was evaluating 16 studies and 18 reviews. The paper used the Life Cycle Impact Assessment technique taking into account "environmental impacts of production, transport, processing, storage, waste disposal and other life stages of food production" in order to analyse the three different diets according to greenhouse gas emissions, land use and water consumption. It was concluded that the vegan diet had the least environmental impact, while the omnivorous diet had the greatest. This is in accordance with a multitude of studies showing a clear difference in the respective diets. Animal products are

associated with higher greenhouse gas emissions, land use and water consumption [Chai et al., 2019].
The feeding of human-edible food to livestock results in a loss of available protein of 51 g per capita per day, globally. This is more than the daily requirement for protein [Berners-Lee et al., 2018].

3.4 Sustainable Livestock Systems

As already discussed in the previous sections, the consumption of animal products is much more strongly linked to increased resource consumption and environmental degradation than the consumption of vegetal products. This connection holds true for the current situation, but is not necessarily true if alternative concepts are taken into account that are based on different concepts than conventional models.
Animal protein can be produced in a way that does not rely on arable land and hence does not compete with crop production. It can even contribute to increased food security by optimizing the recycling of food waste and fulfil a variety of ecological functions improving the efficiency of cropping systems. The critical aspect is the nutrition of the livestock, which is determining the sustainability of the consumption of animal products. Judging the sustainability of any livestock system without taking into account the origin and type of feed is fundamentally flawed.
In order to maximize the sustainability of livestock production, it must be integrated into the whole process of food production and waste management as much as possible.
Röös et al. (2016) proposed a concept of sustainable livestock production for Sweden, where only ecological leftovers were fed to livestock, meaning the animals were pasture raised or received by-products from food processing, but no animal feed was grown on arable land. Applying this scenario would result in reduced consumption of animal products and substantially reduced environmental impact.
Schader et al. (2015) were modelling a similar approach of sustainable livestock production as a global scenario for 2050 compared with a reference diet. Animals were only fed from grassland or by-products, as a consequence animal product consumption was reduced, while environmental impacts, such as arable land occupation, nitrogen and phosphorus surplus, pesticide and freshwater use were lessened.
Van Zanten et al. (2015) calculated that if livestock was fed with co-products, food waste and grass-based systems only, without competing with cropland, the amount of animal protein consumed per person would be 21 g per day, which is about two thirds of current global average consumption [van Zanten et al., 2016].

3.4.1 Food Waste and By-Products Recycling

Global food loss is substantial and consists of losses during agricultural production, livestock production, handling, storage and transportation, consumer waste and overconsumption. The proportion of harvested biomass from agriculture that is actually consumed as human food is just 24.8 %, globally.
Food processing comes with a loss rate of 24.2 % of DM (14.7 % for energy and 33.4 % for protein) and consumer waste with a loss rate of 9.0 % of DM (8.6 % for energy and 9.0 % for protein) [Alexander et al., 2017]. It is estimated that about one third of globally produced food is wasted or lost. Most common food waste management strategies include land filling, composting, anaerobic digestion and incineration. For the United States the proportion of food waste that enters landfills lies between 54 and 97 %, while less than 3 % is composted and less than 2.1 % is anaerobically digested for

biogas production [Kibler et al., 2018].
Feeding of food waste to animals is common in a number of states in Asia. For instance in Japan and South Korea, 35.9 % and 42.4 % of food waste is fed to livestock, mostly pigs. The food waste is sterilized through heat treatment to decrease contamination risks and possibly dried prior to feeding. In the European Union, for most types of food waste, feeding to livestock is illegal due to purported contamination risks. If food waste would be used for pig feed at similar rates as in Japan and South Korea, it would support 20 % of EU pork production [Salemdeeb et al., 2017]. In the US, the usage of food waste for growing pigs is permitted in 28 of the 50 states [Mo et al., 2018]. The use of processed animal protein such as meat and bone meal is prohibited in the European Union, due to concerns over the spread of prion disease [Mo et al., 2018].
Hossein and Dahlan (2015) investigated the substitution of free-range village chicken formulated feed with dehydrated processed food waste. They found it could be utilized at a substitution rate of 20 % without affecting growth performance [Hossein and Dahlan, 2015]. The utilization of food waste as animal feed is better suited for fish than for pigs or poultry, as the transmission of foot and mouth disease, swine fever, highly pathogenic avian influenza, and transmissible spongiform encephalopathies through intra-species recycling (feeding pork to pigs) is recognized as a potential safety concern. However, these four infectious diseases are not related to fish. Feeding fish to fish, even the same species is not perceived as a safety risk.
Food waste can either be heat-treated and dried into a powder or fed to black soldier fly larvae that are harvested, dried and ground. Even though the yield for the first treatment option is substantially higher, the conversion to larvae powder is preferred, as authorization for export and general acceptance are higher with an estimated market value price per ton at least 10 times higher than for food waste powder. The usage of food waste powder as substitution of fishmeal in the diet of several fish should not exceed 20 %, while insect powder would be in the range of 17 - 30 %, according to various feeding trials [Cheng and Lo, 2016].
According to estimations, over 50 % of total fish capture is not used as food. Fish waste that includes bones, intestines, heads and tails can be used to produce fishmeal. This fish waste derived fishmeal would amount to about 50 % of the already used fishmeal in China [Mo et al., 2018].
Fruit and vegetable wastes and by-products from fruit and vegetable processing accumulate at 55 gt per year for India, the Philippines, China and the USA. For the most part they are being disposed of in landfills or rivers. The livestock sector however, provides an opportunity to recycle these resources by including them into the diet of different species of livestock. Wastes such as apple pomace, banana peels or citrus pulp can be incorporated into the diet of ruminants as well as non-ruminants at levels ranging from 10 up to 30 % without compromising growth performance [Wadhwa and Bakshi, 2013].
Van Zanten et al. (2013) calculated that just by using co-products and food waste from an average vegan diet, 71 g of pork containg 14 g of protein can be produced per person per day.

3.4.2 Utilization of Non-Arable Land

The current global arable land covers 1.4 billion ha of land. The remaining potential for available arable land is estimated at 1.4 billion ha, without taking into account forests, built-up areas and protected areas [Alexandratos and Bruinsma, 2012]. Permanent meadows and pastures are comprising 3.3 billion ha [FAO, 2013], while the majority of this land is not suitable for use as arable land.

Ruminants play an important role in global food security, as they have the ability to turn pastureland into human edible food. As most pastureland is not suitable for crop production, ruminants that are 100 % pasture raised do not present a competition for human food production. Rather do they enable the production of food on land areas that would otherwise hardly produce any food at all [Schader et al., 2015].
According to Poore & Nemecek (2018), global beef production (from beef herds) has a protein productivity of 6.1 kg/ha/y [Poore and Nemecek, 2018]. According to the compiled data of Clark & Tilman (2017), the protein productivity of grass-fed beef according to several different sources averages at 8.32 kg/ha/y, while grain-fed beef averages at 11.28 kg/ha/y [Clark and Tilman, 2017].
Through the combination of pasture and trees, called silvopastoral systems, the productivity of conventional grazing systems can be massively increased. In Australia, the combination of grassland with forage trees, the leucaena-grass-system is very popular. On 150,000 ha in Queensland, this system achieves a live weight productivity of 250 kg/ha/y (equal to a protein productivity of 25 kg/ha/y, assuming an edible portion of 50 % of the animals' live weight and a protein content of 20 %). The same system achieves a live weight productivity of 1,000 - 1,500 kg/ha/y (equal to a protein productivity of 100 - 150 kg/ha/y, under the same assumption) when irrigation is provided [Shelton and Dalzell, 2007].
A comparison between conventional grazing systems and intensive silvopastoral systems in Australia, Mexico and Columbio showed an increase in meat productivity of 304 %, 332 - 983 % and 1,353 - 2,257 %, respectively. The leucaena-grass-system is based on the Leucaena tree (*Leucaena leucocephala*), a nitrogen fixing forage tree that provides many ecological services and boosts beef production capacity tremendously, compared to grassland only. This is mainly due to increased forage production and drought resistance. The combination of trees and grazing animals, known as silvopasture systems, has enormous potential to boost global grazing system productivity, while minimizing the need for nitrogen fertilizer due to increased nitrogen fixation capacity and protecting the soil against erosion through more extensive root systems [Cuartas Cardona et al., 2014].

3.4.3 Other Benefits of Integrated Systems

The integration of livestock into crop production can have a multitude of benefits ranging from nutrient cycling and conservation, utilization of crop residues, vegetation and weed management to economic stability through diversified income. For instance Clark and Stuart (1996) showed that geese would greatly reduce insect damage on apple trees and triple the yield of potatoes by selective weeding when they were integrated into a polyculture system and compared to a control system without geese.
Mixed crop-livestock systems are especially important in developing countries. Livestock is used to plough the fields, the manure is used to fertilize the land and the crop residues are fed to livestock. Additional income from livestock products may attenuate harvest losses in dry years. In India, the employment of improved dual-purpose varieties of sorghum and millet have had substantial improvement in livestock efficiency by increasing milk yield from cows and buffaloes by 50 %, while grain yield was unchanged. The dual-purpose varieties are producing more crop residues, directly benefiting integrated livestock production. Globally, 50 % of all produced grain comes from mixed systems already [Tester and Langridge, 2010].
Rice-duck-systems are a traditional practice in China. The ducks are beneficial for the rice cultivation as they serve as a biological pest control by feeding on golden apple snails

that can otherwise be detrimental to the rice crop. On the Philippines, there are rice systems in combination with ducks and fish. The fish also serve as pest control feeding on snails and weeds. In a field trial it was shown that the combination of fish and conventional rice cultivation (using pesticides) increased the rice yield by 10 % compared to conventional rice cultivation alone. Without the use of any pesticides, the combination of rice with fish and ducks resulted in a 25 % rice yield increase compared to conventional rice cultivation using pesticides. Additionally it resulted in an income increase of 1,100 % in the third cropping, due to the selling of duck eggs and fish together with more rice and cutting out the pesticides. See section 4.2.4 for further details [Cagauan et al., 2000].
Maughan et al. conducted field trials where they compared corn monoculture systems with crop-livestock integrated systems. In the latter, corn was grown, while the crop residue was left on the field to be grazed by cattle. Additionally winter cover crops were included after the corn harvest that were also grazed by cows. In 4 years of the experiment, the yield of the integrated system was between 5 and 15 % higher than the monoculture system. Soil quality was improved in the integrated system, as total nitrogen content, total carbon content and soil microbial biomass carbon were increased with larger soil aggregates throughout the study period at different soil depths [Maughan et al., 2009].
Silvopasture systems can also be used to combine livestock with timber production. Clason (1995) showed that a pine timber pasture exceeded the internal rate of return in comparison to an open pasture. Both trees and pasture would benefit from fertilization, while the cattle would keep the grass beneath the trees short, resulting in increased timber production of 5.4 m^3/ha more than the untreated pine plantation.

3.5 Grains vs. Greens: Health Consequences for Humans and Animals

The intensification of livestock production changed the nutritional profile of animal products, by heavily relying on grains and soybeans as the basis of most concentrate feeds. Among the most drastic changes is the impact on the fatty acid (FA) profile, that is being passed on through the food chain. The amount and type of fat in the human diet and especially the content and the ω-6/ω-3 ratio has profound effects on health and cognitive function. The ω-6/ω-3 ratio in animal products is mainly determined by the ratio in the feed, apart from other factors as well.

Linoleic acid (LA) and Alpha-Linolenic acid (ALA) are the only two FAs that are considered essential for human beings. This means they cannot be formed out of other FAs and therefore must be present in the diet. Several other FAs are considered conditionally essential, meaning they can be formed out of the two essential or other FAs, but the conversion rate might not be enough to provide optimal levels at all times.
LA and ALA belong to the group of polyunsaturated FAs that all have at least two carbon-carbon double bonds. Polyunsaturated FAs are either ω-6 or ω-3 FAs. LA is an ω-6 FA and ALA is an ω-3 FA; the number is indicating how many carbon atoms the first double bond is away from the methyl carbon end of the FA molecule. Apart from polyunsaturated FAs, there are also monounsaturated FAs that have only one double bond, consisting of ω-9 and ω-7 FAs and there are saturated FAs, containing no double bond. A fat molecule consists of one glycerine molecule bound to three FAs and is also called a triglyceride. FAs might also be bound into phospholipids or cholesteryl esters [Whitney et al., 2010].

3.5.1 The ω-6/ω-3 Ratio and Human Health

The ω-6/ω-3 ratio in the human diet plays a crucial role in health and cognitive function. While this ratio has been balanced for the most part of human evolution, estimated at 0.79:1 in paleolithic times, today it lies at about 20:1 in modern civilisation. The ratio for different populations is depicted in table 3.1.
While this shift in dietary fats has only been very recent, there has not been any time for the human genome to adapt, so it is still depending on a balanced ω-6/ω-3 ratio for the most part. The more the ratio is in favour of ω-6 FAs, there more pro-inflammatory effects can be witnessed. Several chronic diseases are associated with an excess of dietary ω-6 FAs in relation to ω-3 FAs, such as coronary heart disease, cancer, obesity, diabetes and the metabolic syndrome. Both LA and ALA represent building blocks for metabolic products such as prostaglandines, thromboxanes and leukotrienes having hormone-like effects that are influencing inflammatory processes in the body. While these metabolic products derived from ALA, an ω-3 FA, tend to express anti-inflammatory effects, the ones derived from LA, an ω-6 FA tend to have pro-inflammatory effects. An excessive intake of dietary LA in relation to ALA leads to an excessive production of pro-inflammatory metabolites, as both FAs are competing for the same enzymes that catalyse the conversion to the metabolically active compounds [Simopoulos, 2016].
However, the prognostic value of the ω-6/ω-3 ratio has also received criticism for not

Table 3.1: The ω-6/ω-3 ratio in different populations, after Simopoulos (2016).

Population	**ω-6/ω-3 ratio**
Paleolithic	0.79
Greece prior to 1960	1.00 - 2.00
Current Japan	4.00
Current India, rural	5 - 6.1
Current UK and northern Europe	15.00
Current USA	16.74
Current India, urban	38 - 50

being specific enough and oversimplified. The ω-6/ω-3 ratio differs to a great extent in different tissues and lipid compartments throughout the body, while different types of both ω-6 and ω-3 FAs with different chain lengths exert different degrees of effectiveness not accounted for when calculating the ratio.
Harris (2018) states that ω-6 FAs such as LA failed to produce any inflammatory response in medical trials. He continues that for the most part, the better predictor of health is the percentage of ω-3 FAs in the red blood cell membranes, more specifically the long chain ω-3 FAs Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA), while the percentage of ω-6 FAs or the rω-6/ω-3 ratio is of minor importance.
For instance, one research review found a correlation between the occurrence of Attention Deficit Hyperactivity Syndrome in children and the ω-6/ω-3 ratio in different lipid fractions. The ratio was on average 43.8 % higher in the study group compared to the control, involving 485 subjects. However, the ratio of Arachidonic acid (AA), a long-chain ω-6 FA to EPA, a long-chain ω-3 FA was on average 53.9 % higher in the study group than in the control group, involving 279 participants. While both ratios showed a clear association, the long chain FAs clearly showed a higher correlation in subjects with Attention Deficit Hyperactivity Syndrome [LaChance et al., 2016].
Fossil records are showing that at least 2 million years ago, *Homo habilis* populations were already living around lakes and other water bodies and consuming fish and shell-

fish on a regular basis. This behaviour is seen as the precondition of the start of the evolutionary development of the modern human brain.

The capacity to build DHA out of other ω-3 FAs with a shorter chain length is very

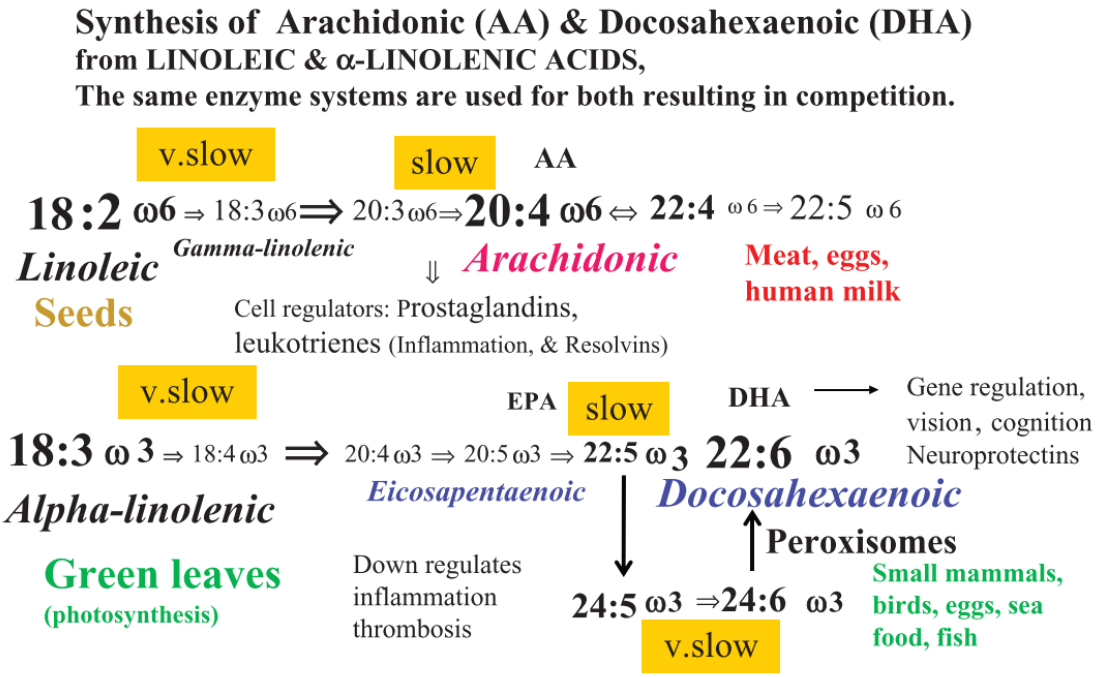

Figure 3.12: Essential fatty acid metabolism, from Crawford and Broadhurst (2012)

limited in humans and also competes with the elongation process of the shorter ω-6 FAs, see figure 3.12. Infants that are not supplied with sources of DHA only acquire about half the amount of DHA in the brain compared with infants that receive adequate amounts of DHA in their diet. DHA is a main structural and functional component of the human brain [Cunnane and Crawford, 2014].

EPA and especially DHA are mainly found in the marine food chain, as they are abundantly produced by microalgae constituting the basis of the food chain of all marine ecosystems. The consumption of foods high in DHA were crucial for the evolutionary development of the hominid brain. It was suggested that the evolution of the large human brain was depending on the close proximity to coastal areas, so that fish and shellfish, the most concentrated sources of DHA, could be regularly consumed [Crawford et al., 1999].

Crawford and Broadhurst (2012) are linking the lack of long-chain ω-3 FAs in the modern diet to the substantial increase of brain disorders that has overtaken all other health costs in the European Union. Human mental ill-health is part of a global crisis and needs to be addressed by fixing the global food system.

3.5.2 The ω-6/ω-3 Ratio in Animal Products

As corn, wheat and soybeans constitute the basis for the great majority of concentrate fed to livestock, the FA profiles of those seeds are reflected in the animal products [Berners-Lee et al., 2018]. Agriculture has drastically changed the human diet, as well as the diet of livestock. Grains and other seeds are typically high in the ω-6 FA LA and low in the ω-3 FA ALA. Compared with the majority of seeds it is the other way around

with most green plant tissues like grasses and leaves, so that the FA profiles from wild animals differ substantially from livestock receiving concentrate feed.
For instance wild trout, eel and salmon have an ω-6/ω-3 ratio of 0.14:1, 0.2:1 and 0.09:1 respectively, while the cultured specimens have higher ratios of 0.5:1, 0.5:1 and 0.17:1 respectively. While an egg from free ranging chickens (probably meaning unfed in this context) has a ratio of 1.3:1, a conventionally produced egg has a ratio of 19.9:1. The free range egg contains 6.6 mg of DHA per g of yolk, while the conventional one only contains 1.1 mg. The same finding holds true for all animal products to a varying extent [Simopoulos, 1999].
There is a considerable amount of research regarding the manipulation of the FA profile of animal products by the utilisation of certain additives to the animal feed, such as flax seed (or lin seed), rape seed, fish meal, fish oil, algae and derivatives thereof. These additives are high in either ALA or EPA and DHA and comparatively low in ω-6 FAs. Different animal products possess very different potentials for having their content of ω-3 FAs increased. Polygastrics (or ruminants) for instance hydrogenate the polyunsaturated FAs in the feed into saturated FAs using bacteria in their digestive tract. Hence, they have a very low content of polyunsaturated FAs in their body fat as well as in the milk fat, so even when the ratio is changed, the effective outcome is very little. The strongest effect is seen in the production of eggs and fish, less so in poultry and pigs, with the least effect seen in polygastrics.
By using extracts of lin seed and rape seed under ideal conditions, the amount of ALA can be increased 40-fold in eggs, 10-fold in chicken, 6-fold in pork and 2-fold in beef. By adding fish or algae products, the amount of DHA can be increased 20-fold in salmon, 7-fold in chicken, 6-fold in eggs and 2-fold in beef. The additional feeding of ALA-rich products raise ALA in the respective animal products, but can also raise the DHA levels, as all animals are capable of transforming ALA into DHA to varying degrees. Chickens that were being fed lin seed oil had 8.1 times more ALA and 2 times more DHA in the fat of the legs. When being fed fish oil, they had 1.3 times less ALA, but 7 times more DHA. Similar results can be seen for the production of eggs in table 3.2. Burre (2005) claims that production costs for ω-3 eggs increase only by 1 % over conventional eggs by incorporating linseed into the concentrate feed. The consumer price is raised by 4 %, while the nutritional quality of the egg is enhanced and closer to the natural form.

The difference between grass-fed and grain-fed beef in terms of FA composition has

Table 3.2: The effect on the FA composition of eggs from laying hens that were fed with different types of oils in their diet, after Bourre (2005).

	control	**fish oil**	**linseed oil**	**soybean oil**	**safflower oil**
ALA % FA	0.4	0.4	9.4	1.7	0.06
EPA % FA	-	2.1	0.3	-	-
DHA % FA	0.6	6.3	2.2	1.8	0.2
Total ω-3 % FA	0.6	10.1	12.6	3.1	0.3
Total ω-6 + ω-3 % FA	18.8	19.7	32.3	28.3	36.2
ω-6/ω-3 ratio	17.8	1.0	1.6	8.1	120

been the subject of several studies. Grass-fed beef has considerably more ω-3 FAs than grain-fed beef, while the difference in ω-6 FA content is much smaller. While commercial laying chickens only derive a small percentage of their diet from pasture, cows as polygastrics are adapted to feed on grass only, which explains the impact of the different feeding regimens [Daley et al., 2010].

An analysis of the FA profiles of different production types of eggs revealed that there was no difference in the ω-6/ω-3 ratio in organic to conventional eggs. Even three subtypes of conventional eggs, "barn-laid, free range and cage eggs" showed very little differences in the ratios. However, eggs from hens that were fed flax seed or fish oil, marketed as "ω-3 eggs" had a considerably lower ratio and more than twice as much DHA as the other eggs. Access to pasture therefore has very little, if any effect on the polyunsaturated FA profile in eggs, as still, the composition of the concentrate feed is the determinating factor of the FA composition of the egg [Samman et al., 2009].
Another study showed a strong impact of pasture access on the FA profile, raising both ALA and DHA compared to caged hens. However, the pastured group was severely restricted in their concentrate rations, while the caged group had *ad libitum* access. Hence, altering the FA profile of eggs by access to pasture is unlikely without the restriction of the concentrate feed for the chickens [Karsten et al., 2010].

3.6 Conclusion

The majority of global livestock production is depending on feed produced on arable land and thereby competing with human edible crop production. Livestock productivity increases through intensification will increase this competition, calling for a reduction in the consumption of animal products in order to alleviate pressure on global agricultural resources.
However, while this approach may extend the time until systemic agricultural collapse, it does not attack the problem at the root. Several strategies for sustainable livestock production exist with huge global potentials that remain largely untapped, like recycling of food waste, silvopastoral systems and other integrated systems making use of crop-livestock synergies.
In this context, livestock systems are not necessarily a threat to food security, but can improve it, by complementing crop production and increasing resource efficiency.
The choice of animal feed has a great impact on the quality, especially the FA profile of animal products. Intensification attempts lead to higher feeding rates of seed crops high in ω-6 FAs and low in ω-3 FAs. This trend in livestock production has negative human health impacts.
The next chapter will deal with the potential of floating plant-based systems that not only allow the production of livestock feed on non-arable land, but produce far more biomass and protein per area than conventional crops.

4. Floating Plants for Sustainable Protein Feed Production

Floating plants are fresh water aquatic macrophytes that are freely floating on the water surface without being attached to the pond bottom, like water lilies for instance. There are many types of floating plants from different plant classes. This thesis will only focus on duckweed, a whole plant family and *Azolla*, a genus.

4.1 Duckweed

Figure 4.1: *Lemna gibba* (bigger fronds) and *Spirodela polyrrhiza* growing together in a pond.

Duckweeds or waterlens comprise a plant family (Lemnaceae) with the five genera *Lemna, Landoltia, Wolffia, Spirodela* and *Wolffiella* containing 38 species in total. They are aquatic freshwater macrophytes that can be found worldwide, often growing in thick green mats covering freshwater bodies that are high in nutrients. Duckweeds are known for their extreme reduction and miniaturization of organs, see figure 4.1. They do not posses any stems or leaves, but are organized in fronds, sometimes even lacking roots. They float on the surface of water bodies or some species float just below the surface. Certain duckweed species comprise the world's smallest angiosperms (flowering plants) with a diameter of just 0.3 mm at maturity. Duckweeds can be found throughout the world in most climate zones in all kinds of freshwater bodies, preferably with little to no current [Les et al., 2002]. Several duckweed species that are adapted to cold climates form starchy fronds, called turions that sink to the pond bottom over winter and come back to the surface when temperatures are rising again in spring [Skillicorn et al., 1993].

4.1.1 Productivity and Cultivation

Duckweeds are capable of doubling their biomass every few days, resembling rather bacteria than plants in this regard, making them the world's fastest growing higher plants. They reproduce vegetatively and sexually, however flowering is rather rare and unpredictable. Their extreme productivity and ease of cultivation makes them interesting for many applications including wastewater treatment, bioenergy production, carbon dioxide capture and feed production.

Productivity
Reported yields of duckweed cultivation under field conditions go up to 73 t DM/ha/y (tons of dry matter per hectare per year). Under laboratory conditions, extrapolated yields of far beyond 100 t DM/ha/y have been achieved, although the transferability to field conditions is questionable, however [Leng, 1999]. Mohedano et al. (2012) reported a productivity of 68.8 t DM/ha/y for *Landoltia punctata* grown in a 153 m^2 outdoor pond fertilized with pig slurry biogas digestate in Brazil for a cultivation period of one year. There is also a report of the duckweeds *Spirodela oligorhiza* and *Spirodela polyrhiza* yielding 500 kg DM per day per hectare during the growing season of 9 months in fertilized outdoor ponds in Louisiana. This would be an extrapolated yield of 182.5 t/ha/y [National Academies Press, 1976].
Zhao et al. (2014) found that duckweed grew better in mixed species polycultures than in monocultures of individual species. For instance a mixture of *Landoltia punctata* and *Lemna minor* had biomass increases that were 17.0 and 39.8 % greater than in the respective monocultures. Polycultures of two or three species tended to have higher nutrient removal rates, higher growth rates, higher starch and crude protein contents than the monocultures in comparison. This might also be an incentive to combine duckweed with floating plants from other plant classes/orders as shown in figure 4.2.

Figure 4.2: A polyculture of different floating plants with several species of duckweed (*Lemna, Spirodela*) and *Azolla filiculoides*, *Pistia stratoites, Limnobium laevigatum*, *Salvinia molesta* and *Ceratopteris thalictroides*.

Harvesting
If maximum biomass yield of duckweed is desired, the harvest needs to be as frequent as every few days to daily. This is necessary to keep the mat density of the duckweed in a certain range. If the mat density is too high, growth will be impeded due to crowding. If the mat density is too low, micro algae will grow extensively below the duckweed mat and compete for nutrients, also resulting in impeded duckweed growth. The ideal mat density for maximum duckweed productivity has been investigated, while the resulting outcomes differ substantially from each other depending on the respective experimental set-ups and range from 400 g/m^2 up to 1,600 g/m^2 [Lasfar et al., 2007]. In theory, the required water depth for duckweed cultivation starts at zero, as it is also capable of growing in mud. However, a certain water depth is needed for proper harvest and also to stabilize the cultivation parameters such as temperature, pH and nutrient levels, as well as providing a buffer for water scarcity in remote locations during the dry season. The ideal water depth must therefore be identified for each individual situation [Leng, 1999].

pH Level
According to McLay (1976) the lower limit, optimum and upper limit for the pH value for three species of duckweed are given as: *Wolffia arrhiza* pH 4 - 5.0 -10, *Lemna minor* pH 4 - 6.2 - 10 and *Spirodela oligorrhiza* pH 3 - 7.0 - 10. Leng (1999) states that duckweeds survive pH values between 5 and 9, while a value between 6.5 to 7.0 is suggested to be maintained, as ammonium is converted to ammonia at higher values, which can be toxic at higher levels.

Temperature Range
Duckweeds are growing at water temperatures between 6 and 33 °C, while the optimum is at around 30 °C.
They prefer full sunlight. In very hot climates however, partial shading of the duckweed might be beneficial in order to keep the water temperature low enough [Leng, 1999]. Lasfar et al. (2007) found a water temperature optimum of around 26 °C for *Lemna minor*, under laboratory conditions.

Nutrients
The two most limiting nutrients for aquatic ecosystems are nitrogen and phosphorus. Hence, for the cultivation of duckweed, most research has only focused on nitrogen and phosphorus levels. Other minerals and trace elements are thought to be present in sufficient amounts through decaying organic matter at the pond bottom [Leng, 1999]. However, this assumption needs to be questioned.
During growth experiments with *Lemna minor* under laboratory conditions, it could be shown that phosphorus-unlimited growth started at around 1.5 mg P/l (=1.5 ppm). The growth rate started to decline at around 20 mg P/l, while at 55 mg P/l, the growth was about 20 % lower compared to the highest values [Lasfar et al., 2007]. Leng (1999) states that maximum tissue P concentrations of duckweed are already achieved with 1 mg P/l in the growing medium, which is suggesting that the minimum requirement for P-unlimited growth lies at around 1 mg P/l.
Lemna minor showed N-unlimited growth under laboratory conditions already at 3 - 5 mg N/l, which started to decline at about 80 mg N/l. At 345 mg N/l growth was reduced by 29 % compared to the highest growth rate values. The nitrogen was mainly provided in the form of nitrate, which is less toxic at high concentrations than ammo-

nium/ammonia [Lasfar et al., 2007]. Leng (1999) suggests an optimal range of ammonia at 20 - 60 mg N/l. While 20 mg N/l is already sufficient for maximum growth, higher values are supposedly needed to maximise the crude protein content of duckweeds, which can fluctuate between 15 and 40 % depending on ammonia levels. The preferred form of nitrogen for duckweed is ammonium, which is in an equilibrium with ammonia. Ammonia can be toxic at higher levels than 60 mg N/l depending on the pH. With increasing pH more ammonium is deprotonated to ammonia.
Mohedano et al. were investigating the growth performance of the duckweed species *Landoltia punctata* in two in-series outdoor ponds, fertilized with pig slurry biogas digestate, so that the first pond always had a higher nutrient load than the second pond. Through one year on average, duckweed biomass from the first pond had a crude protein content of 35 % with a total nitrogen concentration of 44.7 mg N/l in the pond water, while duckweed from the second pond had only 28 % with 14.1 mg N/l. Nutrient concentrations were fluctuating throughout the duration of the experiment with ammonia values reaching as high as 182 mg N/l in the first pond, while a crude protein content of 40 % has also been measured [Mohedano et al., 2012].
Nguyen and Preston (1997) observed both increased biomass yield and protein yield with increasing amounts of nitrogen in the medium of duckweed, as shown in figure 4.3. Appenroth et al. (2017) reported protein content values in duckweed ranging from 6.8

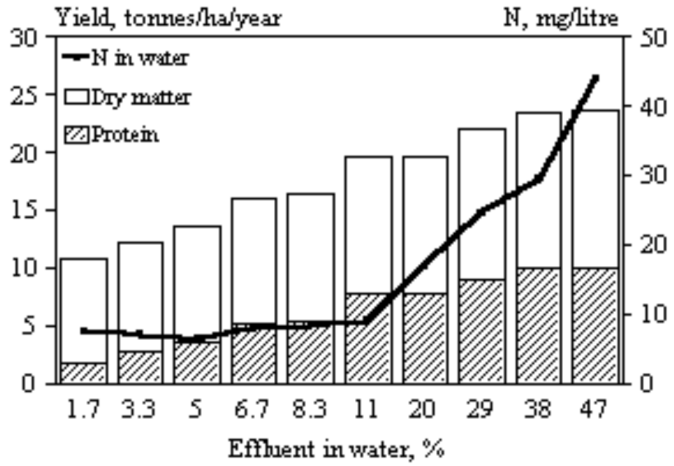

Figure 4.3: Effect of effluent content of pond water on biomass yield of duckweed, after [Nguyen and Preston, 1997].

up to 45 %. High light intensity and nitrate concentrations are purported to lead to high protein contents.
Caicedo et al. (2000) found the highest relative growth rates for the duckweed species *Spirodela polyrrhiza* for ammonia concentrations of 3.5 - 20 mg N/l. Higher ammonia, as well as higher pH values showed inhibitory effects.
Cheng et al. (2002) grew *Spirodela punctata* (or *Landoltia punctata*) in artificial swine lagoon water under laboratory conditions for 20 days. They used 5 different concentrations of ammonium at pH 7, ranging from 63 - 254 mg NH_4^+-N/l. The fastest growth was observed at 240 mg NH_4^+-N/l with 31.92 g/m^2/d (116.5 t DM/ha/y) after a lag period of 96 h, which continued even after N and P was completely absorbed from the medium.
Under laboratory conditions, duckweed can be cultured in Hoagland growth medium [Lasfar et al., 2007]. The Hoagland medium is one of the most popular nutrient solutions used for a great variety of higher plants. It contains all essential plant nutrients, except for nickel [Hoagland and Arnon, 1950]. The essentiality of nickel was only discovered

after the invention of the Hoagland medium. A summary of all essential plant mineral elements is given in table 4.1. A lack of any of these nutrients will inhibit plant growth [Kirkby, 2012]. Skillicorn et al. (1993) argue that duckweed needs a variety of

Table 4.1: Essential plant nutrients and their average concentrations in plant tissue DM sufficient for adequate growth, after Kirkby (2012).

Element	**Chemical Symbol**	**mg/kg**
Nitrogen	N	15,000
Potassium	K	10,000
Calcium	Ca	5,000
Magnesium	Mg	2,000
Phosphorus	P	2,000
Sulphur	S	1,000
Chlorine	Cl	100
Iron	Fe	100
Manganese	Mn	50
Boron	B	20
Zinc	Zn	20
Copper	Cu	6
Nickel	Ni	0.1
Molybdenum	Mo	0.1

trace elements that can be supplied via sea salt. They propose that for each ton of harvested duckweed fresh biomass, 9 kg of sea salt should be dissolved in the pond. The whole fertilizer regimen they used consists of 20 kg urea, 4 kg triple super phosphate, 4 kg muriated potash and 9 kg of sea salt. During their field trials, they harvested 1 ton of fresh biomass every day on one hectare (22 t DM/ha/y), while the pond was receiving the given fertilizer mixture every day.

However, the authors also stated that this fertilizer regimen was inadequate and that further trials are needed to establish the precise nutrient requirements of duckweed and find efficient sources providing the needed minerals.

Urea should also be substituted with ammonium nitrate if available, as urea is quite unstable in water and losses of 50 % N are common. Therefore, 10 kg of ammonium nitrate replace 20 kg of urea according to the authors [Skillicorn et al., 1993].
Haller et al. (1974) found that *Lemna minor*, cultivated in salt concentrations ranging from 0.17 to 5.00 ppt, grew best at a salt concentration of 1.66 ppt (=1,660 mg/l). However, as seawater from the Atlantic Ocean was used in the experiment, the increase in growth rate might be due to other elements besides sodium chloride.
Appenroth et al. (2017) were cultivating duckweed in a "modified Schenk-Hildebrandt medium", which also contained all essential plant nutrients except for nickel, but with added iodine. Naumann (2007) grew duckweed (*Lemna minor*) in artificial medium (Steinberg medium) with 10 different heavy metals added individually in different concentrations and saw an increase in growth rate with arsenite, chromate, cobalt, copper, nickel and zinc in lower concentrations (0.1 - 10 µM). While zinc, copper and nickel are essential plant nutrients and hence would be expected to increase the growth rate, arsenite, chromate and cobalt are not considered essential. It is possible that a variety of elements not essential might still be beneficial for the growth of duckweed.

4.1.2 Suitability as Livestock Feed

Several nutritional parameters of duckweed, as well as *Azolla* in comparison to soybeans, wheat, maize and alfalfa can be seen in table 4.2.

Table 4.2: Some nutritional parameters of duckweed, *Azolla*, soybeans, maize, wheat and alfalfa, after [Heuzé and Tran, 2015] [Tran, 2015] [Heuzé et al., 2017b] [Heuzé et al., 2017a] [Heuzé et al., 2015] [Heuzé et al., 2016].

	Duck-weed (fresh)	*Azolla* (*Azolla spp.*, fresh)	Soy-beans (all)	Maize grain (North America)	Wheat grain	Alfalfa (aerial part, fresh)
Main Analysis						
DM (% as fed)	5.6	6.7	88.7	87.2	87	19.9
Crude Protein (% DM)	29.1	20.6	39.6	9.5	12.6	20.6
NDF (% DM)	40.1	43.8	13.2	11.9	13.9	39.3
Ether Extract (% DM)	6.1	3.8	21.4	4.1	1.7	2.9
Ash (% DM)	15.9	15.9	5.7	1.4	1.8	11.5
Starch (% DM)	n.a.	4.1	6.4	73.1	69.1	0.3
Total Sugars (% DM)	n.a.	n.a.	8.7	3.1	3.2	5.6
Minerals						
Calcium (g/kg DM)	23.3	11	3.2	0.2	0.7	19.4
Phosphorus (g/kg DM)	5.7	6.1	6.1	2.9	3.6	2.5
Potassium (g/kg DM)	42.9	17.4	18	3.5	4.6	22.4
Sodium (g/kg DM)	1.4	9	n.a.	n.a.	n.a.	0.5
Magnesium (g/kg DM)	n.a.	5	2.4	1.2	1.2	2.8
Manganese (mg/kg DM)	1,723	762	29	13	40	76
Zinc (mg/kg DM)	75	38	43	23	31	43
Copper (mg/kg DM)	20	16	19	2	6	13
Iron (mg/kg DM)	n.a.	3,900	121	21	78	387
Amino Acids						
Phenylalanine (% protein)	4.1	5.4	5	4.8	4.5	4.5
Valine (% protein)	4.3	5.5	4.7	4.9	4.3	6.4
Threonine (% protein)	3.1	4.7	3.9	3.5	2.9	4.2
Tryptophan (% protein)	n.a.	1.8	1.3	0.7	1.2	1.5
Methionine (% protein)	0.8	1.4	1.4	2.1	1.6	1.6
Leucine (% protein)	6.6	8.4	7.5	11.8	6.5	6.5
Isoleucine (% protein)	3.6	4.5	4.5	3.6	3.4	4.1
Lysine (% protein)	3.9	4.7	6.2	3.1	2.9	5.5
Histidine (% protein)	1.7	2.1	2.6	2.9	2.3	2

Appenroth et al. (2017) were investigating on the nutritional value of six different species of duckweed grown under laboratory conditions. Duckweeds have a low DM content of just 4 - 8 % of the fresh weight (FW). All nutritional factors are given per DM.

Crude Protein Content and Amino Acids

According to Appenroth et al. (2017) the protein content in the six duckweed species ranged from 20 to 35 %, however this value is greatly influenced by the medium composition and light conditions and can, under extreme conditions, range from as low as 6.8 % up to 45 %. The amino acid composition is comparable to that of other plant protein. In general it compares well with legume flours such as soy or chickpea. There was no case where any critical amino acid was below the recommendations of the World Health Organization.

Leng (1999) states a crude protein content of 25 - 35 % in duckweed grown in natural lagoons and 45 % for enriched culture. The length of the roots of duckweed can be used to judge the protein content, as there exists an inverse relationship, meaning longer roots are associated with lower crude protein content and vice versa. The amino acids Lysine, Methionine, Threonine and Tryptophane are the most limiting amino acids in livestock production and therefore important parameters for the evaluation of protein quality for animal feed [Leuchtenberger et al., 2005].
Leng et al. reported levels of Threonine, Methionine and Tryptophane being higher in duckweed protein than in soybean protein. However, the Lysine content was considerably lower [Leng, 1999]. Skillicorn et al. (1993) claims that duckweed has higher concentrations of methionine and lysine than most plant proteins and rather resemble animal protein in that matter.

Starch Content
Appenroth et al. (2017) found the starch content in 6 duckweed species ranged from 4 to 10 % of DM, however the starch content can reach values over 40 % in the case of nutrient limitation or under high concentrations of salt or some heavy metals, hence the starch content is highly depending on media composition, similar to the protein content. There seems to be a trade-off between protein content and starch content.
Yin et al. (2015) were cultivating *Lemna aequinoctialis* 6000, a duckweed strain that produced a starch content of over 60 % without nutrient starvation. Under specific growth conditions (nutrient starvation) starch contents of up to 75 % in duckweed DM can be realized.

Ash Content
Leng (1999) reports an ash content of 14 - 15 % of DM. He argues that slow growing duckweed has a higher content of fiber, ash, carbohydrates and a lower content of protein. Appenroth et al. (2017) reported an ash content of 16.5 % of DM (only measured in one duckweed species).
The mineral composition is highly dependant on the medium composition, Duckweed can accumulate a number of minerals and trace elements to very high tissue concentrations. Zinc concentrations for instance are reported to vary between 40 and 1,400 mg/kg DM. Duckweed enriched in certain trace elements of high nutritional importance such as selenium or iodine can be easily produced by manipulating the growing medium [Appenroth et al., 2017].

Fat Content
Leng (1999) gives a fat content of 4.0 - 4.4 % in duckweed DM. Appenroth et al. (2017) found the fat content in 6 duckweed species ranged from 4 to 6 % of DM, the most prevalent FA in the analysed duckweed being ALA, a short-chain ω-3 FA. Duckweeds have a high content of polyunsaturated FAs and a very favourable ω-6/ω-3 ratio of 0.25 - 0.61 in relation to the most common feed ingredients that are high in ω-6 and very low in ω-3 FAs (see also table 4.5).
Duckweeds therefore have a great potential to alter the total ω-6/ω-3 ratio in the diet of livestock, changing it towards a more balanced ratio.

Pigments and Antioxidants
Appenroth et al. (2017) stressed the content of lutein and zeaxanthin in duckweed, that is apparently much higher compared to other vegetraian foods, as well as high amounts of tocopherols (vitamin E). Skillicorn et al. (1993) state that duckweeds are particularly

rich in beta carotene and xanthophylls, containing 10 times more total carotenoids than terrestrial plants.

Antinutrients
As found in most plants, duckweeds possess a variety of antinutritional factors that act as a defence mechanism against animals that feed on them. They include trypsin inhibitors, phytates, tannins and oxalates. These substances decrease the availability of nutrients and become increasingly toxic with increasing amounts ingested. Antinutritional factors are therefore limiting the usability of any feed, depending on the animal species and preparation of the feed.
For instance they can be lowered by lacto-fermentation. Experiments showed promising results by fermenting a number of different aquatic plants including the duckweeds *Lemna minor* and *Spirodela polyrrhiza*. All measured antinutritional factors were reduced substantially by lactic acid fermentation using a *Lactobacillus reuteri* strain with added molasses (150 g/kg) at 25°C for 60 days, as shown in table 4.3. The aquatic plants were dried and mixed with fresh plant matter to get a a resulting total DM content of 350 - 450 g/kg. The fermentation process also decreased the fiber content and increased the protein content of the duckweed, making it more valuable as livestock feed [Cruz et al., 2011]. Fasakin (1999) showed that through processing duckweed (*Spirodela polyrrhiza*)

Table 4.3: Concentration of antinutritional substances in raw and fermented aquatic macrophytes, after Cruz et al. (2011).

Antinutritional substances	***Lemna minor***		***Spirodela polyrhiza***		***Azolla filiculoides***	
	raw	fermented	raw	fermented	raw	fermented
Trypsin inhibitor (mg/g)	2.31	0.5	0.8	0.17	1.86	1.37
Phytates (% phytic ac.)	0.32	0.12	0.25	0.11	0.15	0.15
Soluble tannins (%)	0.3	N.d.	1.31	N.d.	0.44	N.d.
Condensed tannins (%)	N.d.	N.d.	3.87	N.d.	N.d.	N.d.
Oxalates (%)	2.02	0.04	0.1	N.d.	1.67	0.19

biomass into a "leaf protein concentrate" through protein extraction, the amounts of cyanide, tannin and phytic acid could be lowered, while the protein content was more than doubled, making it more suitable as livestock feed.

4.1.3 Feeding Trials

Feeding trials are used to determine the effect of certain diets or components thereof on animal productivity. The two most important aspects determining the profitability of most livestock production systems are the feed conversion ratio (FCR) and the price of the feed component. For instance, feeding cost makes up about 70 % of total production costs in the poultry industry [Ara et al., 2015]. For the production of Nile tilapia it can be 40 to 70 % of total production costs depending on the culture system [Tavares et al., 2008].
The FCR states how much feed (as DM) is needed to produce a certain amount of body live weight gain in the animal (as FW). This value can also be given for the amount of eggs or milk. The lower the ratio the more efficient the conversion of feed to animal product. In order to find out the best inclusion percentage of an alternative feed component, the effect on the animals' productivity and the total feed cost has to be considered. As shown in an example in figure 4.4, even though the livestock productivity is best at 10 % inclusion, an inclusion of 20 % results in maximum overall profitability due to

the reduction in feed costs. The alternative feed component can be included into the standard diet by replacing only a certain component or by replacing a fraction of the whole formulated diet.
Due to the high productivity and ease of cultivation, duckweed is a cheap alternative source of protein. Skillicorn et al. (1993) gave the cost of production of duckweed including labour, fertilizer etc. at about 32.7 US$ per ton of FW in Bangladesh. Khandaker et al. (2007) stated that duckweed (DM) would cost 12.5 % of mustard oil cake in Bangladesh. Fasakin et al (1999) gave a production cost of duckweed of 41.4 % of commercial fish pellets in Nigeria. Men et al. (2002) calculated with a price for fresh duckweed at 3.7 % of roasted soybeans. Hence, the replacement of conventional protein feed with duckweed lowers the feeding costs.
However, livestock performance has to be studied as too much duckweed in the diet can be detrimental for most animals. Duckweed that is grown on industrial and/or communal wastewater can be contaminated with heavy metals, organic pollutants and pathogens. Wastewater grown duckweed might not be safe as livestock feed, as contaminants travel up the food chain. It should therefore be disposed of in sealed landfills [Iqbal, 1999]. Duckweed has been fed to all kinds of livestock with varying outcomes:

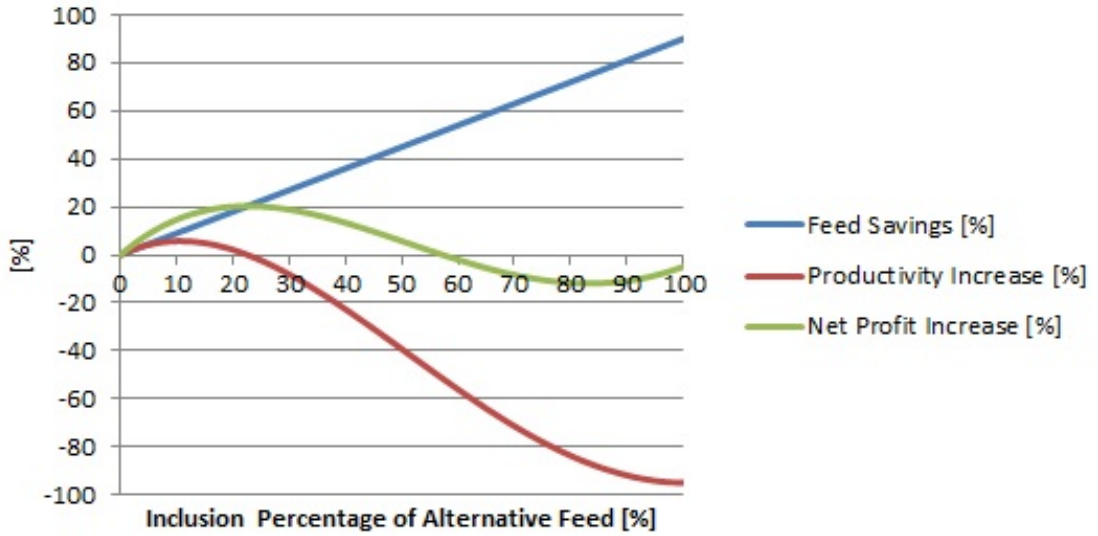

Figure 4.4: Net profit as a function of the inclusion percentage of cheaper alternative feed. While the feed cost savings are increasing with increasing inclusion, the impact on livestock performance can be increased at lower inclusion rates, but decreased with higher inclusion of the alternative feed.

Ruminants
Rusoff et al. (1978) fed fresh duckweed (*Spirodela polyrhiza, Landoltia punctata, Wolffia sp.* and *Lemna gibba*) and maize silage at a ratio of 2:1 of DM to Holstein heifers (150 - 300 kg) for 28 days. The control group received a diet based on corn, concentrate and grass pasture and had half the average daily weight gain compared to the experimental group (450 g/d vs. 900 g/d).
Huque et al. (1996) were investigating on the rumen digestibility and rate and extent of digestion of different species of duckweed in cattle. They fed diets containing 27.9 % duckweed (DM) and concluded that both DM and crude protein of dried duckweed had a high biodegradability in the rumen and may be used as a component of concentrate

feed for cattle.
Belewu et al. (2009) looked at the effects of replacing soybean meal with duckweed (*Lemna gibba*) meal in a mixed concentrate feed for West African dwarf sheep (10 - 18 kg) for 56 days. Replacement was done at 0 (control), 50 and 100 %. The control group and the experimental groups with 50 and 100 % had an average weight gain of 216.07, 208.00 and 90.87 g/d. Both replacement levels showed significant cost benefits due to the low price of duckweed meal.
Babayemi et al. (2006) fed West African dwarf goats with Guinea Grass and substituted it with duckweed at levels of 20 and 40 %. They concluded that duckweed was well accepted by the goats and did not adversely affect feed intake and utilization at 20 %, while feed intake was lowered at 40 % substitution with duckweed. Fresh duckweed was preferred over dried duckweed.

Pigs
Le Thi Men et al. (1997) found that substituting 50 % of the conventional protein sources in the diet of pigs, soybean meal and fishmeal with fresh duckweed resulted in significant improvement of most traits involved in reproductive performance.
Van et al. (1997) proved that rice by-products and protein meals in pig-fattening diets can be completely replaced by ensiled cassava roots and fresh duckweed, with the duckweed supplying 25 % of dietary protein without reductions in growth rate or conversion and resulting in leaner carcasses of the pigs. Piglets (9 kg) were fed a milo (sorghum)-based concentrate, containing 35.95 % soybean meal in the control group. There were 3 experimental groups where the soybean meal was substituted with duckweed at 20, 40 and 60 %. The piglets that received duckweed grew quicker than the control, with increasing inclusion levels of duckweed, weight gain increased as well [Moss, 1999].

Broiler Chicken
Haustein et al. (1994) looked at the effects of different inclusion levels of dried duckweed (*Lemna gibba*) into a corn-based conventional diet of broiler chickens. Average daily weight gain and feed efficiency of the animals was improved at diets containing 10 and 15 % dried duckweed, while at 25 % performance decreased. The authors concluded duckweed could effectively replace soybean meal and fish meal partially and provide high amounts of pigments, which can lower costs for imported protein feed and additives.
Kabir et al. (2005) found a negative impact on broiler chickens when duckweed (*Lemna minor*) was incorporated into the diet at all levels studied (0, 4, 8 and 12 % of DM). Average daily weight gain was reduced with increasing inclusion of duckweed.
Kusina et al. (1999) did experiments on broiler chickens with diets including 0, 10, 20 and 30 % duckweed and came to the conclusion that broiler finisher diets should include 10 % duckweed at most, as higher levels lead to compromised feed intake, live weight gain and feed conversion efficiency.
Ahammad et al. (2003) investigated on the effects of including duckweed (*Lemna minor*) into the conventional diet of broilers at 3, 6 and 9 %, replacing equal amounts of sesame oil cake. Incusion of 3 % improved live weight gain and feed conversion efficiency, 6 % gave further improvements, while 9 % showed a decline compared to the control.

Laying Hens
Chantiratikul et al. (2010) found that duckweed meal (*Wolffia globosa*) could replace soybean meal up to 75 % (at 8.1 % of total diet) without adversely affecting productivity in laying hens. Akter et al. (2011) fed dried duckweed (*Lemna minor*) to laying hens at inclusion levels of 0, 5, 7, 11, 13 and 15 % of the diet. They did not observe any

harmful effects on laying performance up to 13 % and egg quality characteristics were not negatively affected up to 15 %. They concluded that duckweed can be considered as a source of protein and pigments for laying hens.
Anderson et al. (2011) fed laying hens a diet containing 0 and 12.6 % dried duckweed. The diet containing duckweed did not influence the performance of the hens, it did increase the ω-3 content of the eggs, the shell stability and the color score of the yolk, but also increased the percentage of grade B eggs having a reduced appearance over grade A eggs. Haustein et al. (1990) did experiments with laying hens feeding them different levels of duckweed (*Lemna gibba*). TOPAZ laying hens that received 15 % duckweed meal, fully replacing soybean meal, slightly increasing egg laying productivity during 10 weeks of trial.
In another trial, TOPAZ hens that received diets containing duckweed included at 40 %, fully replacing both soybean meal and fish meal, were able to maintain laying productivity at rates similar to the control (slightly less) for 18 weeks, but lost body weight. At 25 % duckweed inclusion productivity was almost the same as in the control group or slightly better, while the hens gained more weight than in the control.
In a trial with HyLine Leghorn laying hens, diets contained 0, 15 and 25 % duckweed meal. Productivity was increased at 15 % during the first two periods at 2 and 6 weeks, but decreased during the last period at 10 weeks compared to the control. At 25 % productivity declined sharply at 6 and 10 weeks compared to the control.
Pigmentation of the yolk was significantly increased with inclusion of duckweed into the diet at all levels [Haustein et al., 1990].

Broiler Ducks
Khanum et al. (2005) studied the intake and digestibility of duckweed under different feeding regimes for growing ducks. The control group received concentrate only, the first experimental group received 50 % of the concentrate and fresh harvested duckweed *ad libitum*, the second group received 50 % of the concentrate and had free access to a pond with duckweed and the third group received no concentrate, but only access to the pond. All ducks of the last group died within 3 weeks. The control group had an average daily weight gain of 9.09 g/d, the first group 6.53 g/d and the second group 6.28 g/d. While growth performance was clearly reduced, carcass parameters were not influenced and feeding costs were decreased substantially.
Khandaker et al. (2007) were replacing mustard oil cake with duckweed meal (*Lemna perpusilla*) at different levels up to full replacement (at 15 % of total diet) in the diet of Jinding layer ducks for 75 days. The duck's productivity declined with increasing levels of duckweed in the diet, however profitability was highest at the highest inclusion level of duckweed, as the savings in feed costs outweighed the loss of productivity.

Laying Ducks
In order to find the best method of feeding duckweed to laying ducks, Indarsih and Tamsil (2012) fed diets containing 20 % duckweed in three different variations: dried duckweed mixed with concentrate, dried duckweed mixed with concentrate and water and fresh duckweed *ad libitum* separately from the concentrate. The second method led to decreased feed intake but increased feed conversion efficiency over the first method and the best laying performance. The third method increased feed intake and produced about the same laying performance than the second method. Duckweed was preferred in fresh form by the ducks and increased pigmentation of the yolks the most.
Men et al. (2002) investigated the effects of replacing soybean meal and fish meal with fresh duckweed (*Lemna minor*) *ad libitum* in the diet of breeding ducks in Vietnam for

3 months. Soybean meal and fish meal were replaced at 0, 25, 50, 75 and 100 %. With increasing replacement, the laying rate dropped from 66.5 % (control) down to 62.3 % for local breeding ducks and from 61.9 % (control) down to 53.5 % in Cherry Valley breeding ducks. Feed cost savings for purchased duckweed were 25 % and if the duckweed was grown on the farm it was 36 %.

Aquaculture

Fish species that have been investigated on their ability to feed on duckweed as part of their diet or exclusively include several carps (*Catla catla, Labeo rohita, Cirrhinus mrigala, Cyprinus carpio, Ctenopharyngodon idella* and *Hypophthalmichthys molitrix*), Thai silver barb (*Barbodes gonionotous*), Nile tilapia (*Oreochromis niloticus*), catfish (*Ictalurus punctatus* and *Heterobranchus longifilis*), snakehead (*Channa striatus*) and Jade Perch (*Scortum barcoo*) [Ansal et al., 2010].

Tavares et al. (2008) did a feeding trial with Nile tilapia (*Oreochromis niloticus*) fingerlings (3.2 g) for 50 days. The fish received a commercial pelleted diet, a diet composed of 50 % commercial diet and 50 % dried duckweed (species not given) and diet composed of 100 % dried duckweed. The total weight gain of the fingerlings was 7.7, 6.0 and 2.9 g, respectively. The FCR was at 1.8, 2.0 and 5.1 respectively. The crude protein content of the used duckweed was given at 38.86 %, a rather high value.

Fasakin et al. (1999) were substituting the fishmeal component of a commercial diet of Nile tilapia (*Oreochromis nilotica*) fingerlings (13 - 15 g) with 0, 5 10, 20, 30 and 100 % with dried duckweed (*Spirodela polyrrhiza*) for 56 days. Weight gain was declining with increasing inclusion of duckweed, however the total cost of production per amount of fish was lowest at 30 % substituion of fish meal with dried duckweed.

Hassan and Edwards (1992) did experiments with Nile tilapia (*Oreochromis niloticus*) being fed with duckweed (*Lemna perpusilla* and *Spirodela polyrrhiza*) only. The aim of the experiment was to find the optimal feeding rate, expressed as DM of duckweed fed as percentage of the total fish body weight per day. In the first experiment *Lemna perpusilla* and *Spirodela polyrrhiza* were being fed to Nile tilapia fingerlings (25 - 28 g) at a feeding rate of 2.5, 5.0 and 7.5 % for 70 days. The FCR ranged from 2.2 to 9.4, while it was increasing with increasing feeding rate. The highest average daily weight gain was achieved at 5 % for both duckweed species, with *Lemna perpusilla* inducing about twice as much weight gain than *Spirodela polyrrhiza.*

In the second experiment, Nile tilapia fingerlings (39 - 44 g) received only *Lemna perpusilla* at feeding rates of 1, 2, 3, 4, 5 and 6 % for 70 days. The FCR ranged from 1.6 to 3.3. Lowest FCR and highest average daily weight gain was seen at a feeding rate of 3 %.

However, both experiments have weaknesses: The two duckweed species *Lemna perpusilla* and *Spirodela polyrrhiza* had a protein content of just 25.3 and 23.8 %, which can be considered low quality. Moreover, the fish tanks were not aerated, so that the duckweed was blocking air exchange at the water surface, which explains the reduction in performance at higher feeding rates. Dissolved oxygen was documented and fell with increasing feeding rates, supposedly leading to low performance and survival rate.

It should be noted that Nile tilapia at market size are usually around 0.5 - 1.0 kg in body weight, while in this experiment fingerlings only up to 111 g were used. Obviously performance characteristics would change at different body weights.

Fasakin et al. (2001) included dried duckweed (*Spirodela polyrrhiza*) into a commercial pelleted diet for Nile tilapia (*Oreochromis niloticus*) fingerlings to substitute the fish meal at 0, 5, 10, 20, 30 and 100 %. The SGR was 2.4, 2.4, 2.4, 2.2, 2.1, and 0.8, respectively. The FCR went from 1.6 (control) up to 4.3 for the 100 % duckweed for fishmeal

substitution. However, the duckweed only contained 25.6 % crude protein, which can be considered low quality.
Pípalová (2003) summarized data from 9 studies and her own concerning growth characteristics of duckweed (*Spirodela polyrrhiza* and/or *Lemna spec.*) fed grass carp (*Ctenopharyngodon idella*) fingerlings. FCRs ranged from 1.60 to 11.60 with an average of 3.69. The SGR ranged from 0.5 to 3.41 %/d, averaging 1.66 %/d.
Yibo et al. (1994) fed duckweed (*Spirodela polyrrhiza*) to grass carp (*Ctenopharyngodon idella*) fingerlings (12 - 13 g) for 3 weeks *ad libitum*. They ate 116 % of their body weight in fresh duckweed on average per day and had a SGR of 3.41 %/d. The conversion efficiency was at 10.62 % of DM. Assuming this value was meant as DM of duckweed per DM of fish, this would translate to FCR of 1.88 assuming 20 % DM content in the fish.
Unfortunately, the great majority of research that has been conducted on feeding fish with duckweed is based on fingerlings, not on market size fish. Obviously, the growth and feeding characteristcs change with the body weight of the fish from a few grams up to over 1 kg making it difficult to judge the feasibility of full scale commercial systems with the limited available research.
Cassani et al. (1982) did research on hybrid grass carp (*Ctenopharyngodon idella* X *Hypophthalmichthys nobilis*) with a fish weight of 1,015 g and 1,033 g that were fed the duckweeds *Lemna gibba* and *Wolffia columbiana*, respectively for 60 days. The FCRs were 6.69 and 3.76 and SGRs were 0.21 and 0.51 %/d achieving 56 and 135 % of the control group that was fed catfish pellets.
El-Shafai et al. (2004) investigated on the effects of replacing parts of the diet of Nile tilapia (*Oreochromis nilotica*) with duckweed. Fingerlings (87 - 91 g) were fed a control diet, a diet containing 20 % dried duckweed, a diet containing 40 % dried duckweed, a diet containing 20 % fresh duckweed and a diet containing 40 % fresh duckweed for 49 days. The FCR was 0.91, 0.98, 1.08, 0.98 and 1.11, respectively. The SGR was 1.51, 1.38, 1.31, 1.44 and 1.33. Productivity declined with increasing amounts of duckweed, while fresh duckweed provided slightly better performance of the fish.
Hasan and Chakrabarti (2009) summarized the findings of 18 studies focusing on fish including Nile tilapia and several carp species that were fed exclusively duckweed, including different species. The FCRs ranged from 1.0 to 6.69 and the specific growth rate (SGR) from 0.21 to 3.88 %/d.
Kabir et al. (2009) compared the productivity of fertilized polyculture fish ponds containing several carp species and Nile tilapia, where one pond received supplementary duckweed and the other pond did not, for 90 days. The feeding of duckweed increased the body weight of the fish by 20 % over the control. Chowdhury et al. (2008) saw a SGR of Nile tilapia (*Oreochromis niloticus*) fingerlings (30 g) in fertilized ponds fed duckweed (*Lemna minor*) at 60 % of the fish body weight (FW basis) daily of 1.16 %/d during 90 days. The control which was not fed duckweed, but sustained on the pond biology only had a SGR of 0.8 %/d.
Skillicorn et al. (1993) states that a grass carp/migral polyculture system produces 1 kg of fish for 10 - 12 kg of fresh duckweed fed, which would translate to a FCR of about 1. However, this does not take into account the plankton that the fish feed on as well. Consequently, the actual FCR is likely at least two times as high.

4.1.4 Integrated Systems

Duckweed can be used to directly recycle nutrients out of anaerobic digestate from liquid manure. Mohedano et al. (2012) demonstrated that duckweed (*Landoltia punctata*)

growing in the biodigester effluent from swine manure, had a nutrient removal efficiency of 98.0 % of total Kjeldahl nitrogen and 98.8 % of total phosphorus. The effluent coming from the digestion of 1 m^3/d of waste from 100 pigs, consisting of manure, urine and leftovers was treated in a pond area of 243 m^2 consisting of two duckweed ponds that produced on average 34.5 kg of fresh duckweed biomass per day that was harvested daily. However, the nutrient load was not only removed by duckweed, but also phosphates were removed by sedimentation and nitrogen was removed by microbial nitrification and denitrification processes. This system proved to be succesful in the on-site polishing and valorization of swine waste digester effluent, while producing valuable biomass high in protein, over the course of one year.

Skillicorn et al. (1993) described an integrated duckweed aquaculture system that was tested as a pilot project in Bangladesh. Duckweed was either grown with manure, mineral fertilizer (NPK and sea salt) or communal wastewater and fed to fish in a separate pond on a daily basis. When wastewater is used, the whole system becomes more profitable as fertilizer or manure does not have to be purchased. However, wastewater does come with the risk of heavy metal contamination, disease transmission and pharmaceutical residues. The fish are fed duckweed only, however they are also able to feed on the pond biology itself, mainly algae and zooplankton. Sophisticated carp polyculture systems have been employed where catla carp (*Catla catla*), mrigal carp (*Cirrhinus mrigala*), silver carp (*Hypothalmichthys molitrix*), mirror carp (*Cyprinus carpio*), grass carp (*Ctenopharyngodon idella*) and rohu carp (*Labeo rohita*) are kept together with the aim of maximizing productivity by making use of their individual feeding habits. Fish that directly feed on the duckweed of the surface are combined with bottom feeders that recycle the undigested parts of the feces of the top feeders. Other fish are filter feeders and feed on the micro algae and zooplankton in the ponds.

A monoculture of duckweed-fed Nile tilapia (*Oreochromis niloticus*) has also been tested successfully using the same set-up, as Nile tilapia are very flexible concerning their feeding habits and can thrive on duckweed, phytoplankton and detritus. The duckweed is grown in shallow ponds, fertilized and harvested every day by hand without processing the duckweed in any way. Fish are harvested about twice per week and sold fresh at the local market. The fertilizer requirements are calculated according to the amount of duckweed biomass harvested daily. This system is very labour intensive, but fish yields were estimated at 10 up to 15 t/ha/y for the carp polyculture. The Nile tilapia monoculture was estimated at 10 t/ha/y. The yields are given for the fish ponds only, without the duckweed production ponds.

The fish ponds were not aerated and dissolved oxygen was the greatest limiting factor for the stocking density of the fish, which was held between 15 and 20 t/ha. The duckweed ponds were producing about 1 (0.5 - 1.5) ton of fresh biomass every day, which is equivalent to 25.5 (13 - 38) t DM/ha/y. Feed conversion was given as 10 - 12 kg of fresh duckweed for 1 kg of fresh fish produced (not considering plankton as feed). Hence, a 1 ha fish pond producing 10 t of fish per year, would need 120 t of fresh duckweed per year grown on about 0.3 ha of pond area. The total area needed to produce 10 t of fish per year would therefore be 1.3 ha, with a productivity of about 7.7 t of fish/ha/y. As the surface area of the fish pond is 3 times bigger than the duckweed pond, it can be assumed that the production of microalgae plays an at least equally important role for the feed supply of the fish [Skillicorn et al., 1993].

Men (1997) describes the traditional smallholder systems of rice cultivation with integrated raising of fish, ducks and duckweed in Vietnam. There, rice is often cultivated together with fish and ducks as part of an integrated system, where both fish and ducks are feeding on rice pests like snails, reduce the occurrence of weeds and fertilize the rice

plants with their litter. Duckweed is grown on wastewater ponds and harvested daily to be fed to the fish and ducks in the rice fields. Additionally, they are being fed food scraps and rice by-products. The fish, mostly Nile tilapia, carp and catfish are a common food in the Vietnamese culture, as are duck eggs and duck meat. The production of animal products contributes significantly to the income of the smallholders in the villages and also fulfils important functions in the rice cultivation. The supplementary feeding of duckweed allows the farmers to sustain on local products almost exclusively by providing protein to their animals.

4.2 *Azolla*

Azolla is a genus of floating aquatic ferns and is also called mosquito fern, duckweed fern, fairy moss or water fern. It contains 7 species that are *A. nilotica, A. caroliniana, A. microphylla, A. mexicana, A. filiculoides* (shown in figure 4.5), *A. rubra* and *A. pinnata*. The taxonomy is however still a matter of debate, as the species tend to be

Figure 4.5: A dense mat of *Azolla filiculoides* growing in an open pond in Northern Germany in June.

difficult to differentiate. *Azolla* has quite a few similarities to duckweed, as both are relatively small (a few mm up to 15 cm) freely floating aquatic macrophytes that are widely distributed in freshwater systems with a preference for nutrient rich water, in which they can proliferate excessively.
Azolla is a fern and produces spores, but mainly propagates vegetatively. *Azolla* is a symbiotic host for the cyanobacteria *Anabaena azollae* and other bacteria that are living inside the fronds of the fern. Through the cyanobacteria, *Azolla* is capable of biological nitrogen fixation, turning elemental nitrogen out of the air into reactive nitrogen. *Azolla* is unique in its efficiency of biological nitrogen fixation, enabling it to grow in water devoid of reactive nitrogen, holding great potential for agricultural purposes [Kumar et al., 2015].

4.2.1 Productivity and Cultivation

Azolla can be cultivated in a very similar way to duckweed. The Natural Resources Development Project (NARDEP) in India described a method for small scale livestock owners to grow *Azolla* as a supplementary feed: A pit is dug out at the size of 2 by 2 m, 20 cm deep, preferably under the shade of trees and covered with a liner (silpauline sheet). Then, 10 -15 kg of fertile sieved soil, 2 kg of cow manure and 30 g of super phosphate fertilizer is mixed with water and filled into the pit. Water is added to a water depth of 10 cm. About 0.5 - 1 kg of fresh *Azolla* is added as a starter culture. After 10 - 15 days, *Azolla* can be harvested daily at 500 - 600 g fresh biomass. Every 5 days, 1 kg of cow manure and 20 g of super phosphate should be added to keep the cultivation productive [Pillai et al., 2002].

Productivity
Purported biomass yields of *Azolla* vary to a large degree.
On a commercial farm in Columbia, *Azolla filiculoides* was grown in outdoor water tanks with a water depth of 40 cm, fertilized with poultry litter at a rate of 10 g/m^2/d. On a total area of 468 m^2, fresh *Azolla* biomass was harvested at about 100 kg a day for 154 days. The extrapolated DM yield was estimated at 39 t DM/ha/y with a yearly protein yeld of 9 t [Becerra et al., 1990].
Liu et al. (2008) report an extrapolated yield of *Azolla* of up to 16.9 t DM/ha/y (assuming DM content of 6 %) under outdoor conditions during a cultivation period of 91 days.
Costa et al. (1999) found the yield of *Azolla filiculoides* growing in a lagoon in Portugal to be up to 27.4 g DM/m^2/d, which is equal to 100.0 t DM/ha/y.
Giridhar et al. (2013) report a daily mean fresh biomass yield of *Azolla* of 800 - 900 g in the season from a surface area of 2.23 m^2 as part of a small scale supplementary livestock feed production for Indian farmers. This extrapolates to 78.6 - 88.4 t DM/ha/y.
Similar to the cultivation of duckweed, if maximum productivity is desired *Azolla* needs to be harvested frequently to keep the relative growth rate high. Brouwer et al. (2017) reported that they started harvesting their culture when *Azolla* started to form a closed mat at a density of 2 - 3 kg of FW or 160 g DM per m^2. After that they proceeded to harvest the biomass at a rate of 33 % twice a week for 138 days and had a yield of 35.5 t DM/ha/y under laboratory conditions.
According to the Natural Resources Development Project (NARDEP) in India, a 2 by 2 m fertilized water surface produces 500 to 600 g of fresh *Azolla* biomass per day. With an estimated DM content of 6 %, this equals an extrapolated yield of 27.4 - 32.9 t DM/ha/y [Pillai et al., 2002].

Temperature Range
Peters et al. (1980) examined 5 species of *Azolla* and found the temperature optimum for *Azolla filiculoides* to be at 25°C and for the other 4 species at 30°C. All species grew well between 20 and 30°C. Watanabe and Berja (1983) grew 4 different species of *Azolla* at 22, 29 and 33°C. The relative growth rate was highest at 29°C for *A. pinnata*, at 29°C for *A. filiculoides*, at 33°C for *A. mexicana* and at 22°C for *A. caroliniana*. For the cultivation of *Azolla* in field conditions, partial shading is of benefit.

Light Requirements
Only about 25 - 50 % of full sunlight is needed for normal growth of *Azolla*. Traditionally, *Azolla* is cultivated in rice fields, where it is shaded by the foliage of the rice plants. The

light saturation points for *A. pinnata* and *A. filiculoides* were at 6,000 lux during spring and increased to 8,000 and 14,000 lux, respectively. Direct sunlight typically reaches values over 100,000 lux at noon [Liu et al., 2008a].
Peters et al. (1980) grew five species/strains of *Azolla* at different light intensities and recorded the doubling times. From 100 to 190 to 420 µmol/s/m^2 growth rate increased, while there was no difference between 420 and 600 µmol/s/m^2 (direct sunlight is about 2,000 µmol/s/m^2). Giridhar et al. (2013) also suggest to use shading for the cultivation of *Azolla*, as it needs only 25 to 50 % sunlight. Wagner (1997) states in most climates, *Azolla* grows best under the shade of vegetation, just like in rice cultivation.

pH Range
Five different species of *Azolla* were shown to have comparable growth rates at pH levels of 5, 6 ,7 and 8. Only at pH 9 was the growth rate clearly inhibited [Peters et al., 1980]. Cary and Weerts (1992) found both *A. pinnata* and *A. filiculoides* grew best at pH levels of 5 and 7.

Nitrogen Fixation
The symbiosis of *Azolla* and *Anabaena azollae* is unique in its efficiency of nitrogen fixation (see figure 4.9). While typical values for legumes reach values up to 400 kg N/ha/y, the *Azolla Anabaena* symbiosis is capable of fixing 1,100 kg N/ha/y [Hall et al., 1995]. Thus *Azolla* together with its symbionts is the most efficient plant in fixing nitrogen out of the air. Herridge et al. (2008) estimated the average global nitrogen fixation rate of the major legume crops, being mainly soybeans at 115 kg N/ha/y. Alfalfa, a common legume forage is estimated at 200, clovers at 150, other legume forages at 100 and mixed grass-legume pastures at 50 kg/ha/y. High yielding legume crops might fix as much as 350 - 400 kg N/ha/y. According to Wagner (1997) *Azolla* can fix 30 - 40 kg of nitrogen per hectare in two weeks in nitrogen-free solution, equal to 782 - 1,043 kg N/ha/y.
Brouwer et al. (2017) reported a nitrogen fixation rate of 1,200 kg N/ha/y for *A. filiculoides* grown under laboratory conditions without any reactive nitrogen in the artificial growing medium.
Cobalt was shown to be essential for the nitrogen fixation of *Azolla* or rather for *Anabaena azollae*. Cobalt was not required, when *Azolla* was provided with sufficient amounts of reactive nitrogen in the medium [Johnson et al., 1966].

Nutrients
Kitoh and Shiomi (1991) grew *A. japonica* in different concentrations and sources of reactive nitrogen ranging from 0 to 20 mM (280 mg N/l). While urea increased the growth at all concentrations, nitrate had a negative effect with increasing concentration and ammonium much more so. Nitrogen fixation rate was reduced with increasing reactive nitrogen concentration in the medium. The reduction was greatest for ammonium, less with nitrate and urea.
Costa et al. (2009) looked at the effects of different nitrogen sources and concentrations on the growth and nitrogen fixation of *A. filiculoides*. The growth rate was enhanced at 5 mg N/l over 0 mg N/l, but declined at 40 mg N/l. At 40 mg N/l, nitrogen as nitrate provided better growth than as ammonium. The nitrogen fixation rate was highest in the medium without any reactive nitrogen and lowest at 40 mg NH_4^+-N/l. *Azolla* was able to take up nitrogen from the medium and still fix it out of the air at the same time.
Kitoh and Shiomi (1991) reported on the effect of different concentrations and sources of reactive nitrogen ranging from 0 to 20 mM (280 mg N/l) on the nitrogen content of *A. japonica*. Nitrate had little effect, while urea increased the nitrogen content with

increasing concentration up to almost 30 % at 20 mM. Ammonium increased the nitrogen content up to a concentration of 5 mM (70 mg N/l) in the medium and at higher concentrations decreased it.
As nitrogen in the solution is not growth limiting for *Azolla*, the main growth limiting nutrient is assumed to be phosphorus. Smallholders in India growing *Azolla* as cattle feed are advised to add phosphate fertilizer together with cow manure to the nutrient solution in order to sustain the growth of *Azolla* [Pillai et al., 2002].
Hasan and Chakrabarti (2009) report a minimum concentration of 0.06 mg P/l for normal growth and 20 mg P/l for optimal growth. A deficiency of phosphorus leads to decreased growth, a red color of the fronds due to increased anthocyanins and curled up roots. Cary and Weerts (1992) grew *A. pinnata* and *A. filiculoides* with 0.01, 5, and 20 mg P/l in the solution at 20, 25 and 30°C. For *A. filiculoides*, 20 mg P/l gave the highest DM yield, while for *A. pinnata*, 5 mg P/l gave the best results for 20 and 25°C and 20 mg P/l at 30°C. The ideal concentration of phosphorus is apparently temperature dependant and increases with increasing temperature.
The most common growth media for *Azolla* are H-40 (Hoagland) and IRRI1/2 (International Rice Research Institute). The phosphorus content in IRRI2 lies at 0.6 mg P/l, while it is 13.9 mg P/l in H-40. They are both devoid of nitrogen, so there is less chance of contamination with algae during the cultivation. All essential plant nutrients are contained except for nickel, and zinc in IRRI1. Additionally, they both contain sodium and cobalt. IRRI2 was shown to give the highest growth rates for *A. filiculoides*, compared to H-40 and IRRI1 [Pereira and Carrapiço, 2009].
Sánchez-Viveros et al. (2010) grew *A. filiculoides* in artificial medium (nutrient solution after Yoshida) with added Arsenate at different concentrations. At a concentration of 5 µM, Arsenate increased the DM yield by 50 %. Only above 20 µM was the growth of *Azolla* reduced.
Azolla tolerates much less salinity than duckweed does. Rai and Rai (1999) found that *A. pinnata* grew better in Hoagland nutrient medium without sodium chloride than at 10 mM (=584 mg/l). However, they were able to demonstrate that *Azolla* was capable of increasing its salt tolerance by cultivating it at sub-lethal concentrations first to accommodate it. After 18 days at 20 mM it was able to grow at 60 mM (=3504 mg/l), while already a concentration of 40 mM killed the plants without prior adaptation.

4.2.2 Suitability as Livestock Feed

Some nutritional parameters of *Azolla* are shown in table 4.2. The low DM content in fresh *Azolla* is similar to duckweed ranging from 2.5 to 7 % [Kumar et al., 2015] and [Hasan and Chakrabarti, 2009].

Crude Protein Content and Amino Acids
Hasan and Chakrabarti (2009) give typical values for crude protein contents ranging from 19 to 30 % for different species of *Azolla*. In general, the protein of *Azolla* is richer in Lysine than conventional plant protein sources.
Costa et al. (2009) showed that the composition of the growing medium of *A. filiculoides* had an effect on the nitrogen content (and thereby also on the crude protein content) in the *Azolla* biomass. In nitrogen free H-40 medium, the biomass had a 5.22 % nitrogen content. With the addition of 5 mg N-NO_3^--/l it was increased to 5.49 %, while the addition of 40 mg N-NO_3^--/l decreased it to 4.38 %. The addition of 40 mg N-NH_4^+/l however, increased it to 6.09 %.
Kumar et al. (2015) analysed the nutritional composition of six *Azolla* species. The

crude protein content ranged from 18 to 26 %.
Liu et al. (2008) mention a crude protein content of 20 - 30 % of *Azolla*,while some new strains can be up to 35 %. They also state that the combined content of the sulphur-containing amino acids Methionine and Cysteine is higher in the protein of *Azolla* compared to alfalfa, soybeans and corn. Costa et al. (1999) reports the composition of *Azolla* biomass from 4 different locations. The protein content ranged from 16.38 to 26.69 %.
Peters et al. (1980) analysed five different species/strains of *Azolla* grown under "optimal laboratory conditions". The nitrogen content ranged from 4.79 to 6.24 %, equal to a crude protein content of 29.9 to 39.0 %. They could show that the nitrogen concentration was also depending on the temperature *Azolla* was grown at. The nearer it was to the optimum where the highest growth rate was observed, nitrogen content was also highest.

Sugar Content
Kumar et al. (2015) found a sugar content of 3.7 to 4.6 % in six different species of *Azolla*.

Ash Content
Hasan and Chakrabarti (2009) give a typical range for the ash content of 14 - 20 % in *Azolla*. Costa et al. (1999) found the ash content ranges from 8.71 to 16.85 %.
Kumar et al. (2015) observed it to be at 14.5 - 24.5 % in six different species and Liu et al. (2008) at 8.71 - 16.85 %.

Fat Content
The crude lipid levels in *Azolla* were observed at 3 - 6 % [Hasan and Chakrabarti, 2009], at 2.0 - 2.9 % [Kumar et al., 2015] and at 3.25 - 5.82 % [Liu et al., 2008].
Costa et al. (1999) found the fat content ranging from 3.25 to 5.82 %.
Bhaskaran and Kannapan (2015) analyzed the FA profiles of 4 different varieties of *Azolla*. The short-chain ω-3 FA ALA was found to be the most prevalent in all 4 species. They also found the long-chain ω-3 FAs EPA and DHA, which are usually not found in plants, but in algae and animal tissue. They might stem from the bacteria living inside the fronds of *Azolla* or possibly from algae that were growing on the roots of *Azolla*. The ω-6/ω-3 ratio ranged from 0.43 to 0.68, which is beneficial for the production of animal products with a balanced polyunsaturated FA profile, similar to duckweed.

Antiutrients
Antinutrients play a huge part in the nutritional properties of plant matter as they can decrease the palatability to herbivores and even have toxic effects. *Azolla spp.* can increase its tissue concentration of deoxyanthocyanin, a plant flavonoid, when stressed by nutrient deprivation, UV-radiation or extreme temperatures, giving the plants a visible red color, as shown in figure 4.6.
Cohen et al (2002) could show that *A. pinnata* increased its deoxyanthocyanin content by 260 % when it was cultured together with tadpoles that were feeding on the plant. The plants also showed a 10 times lower proportion of polyunsaturated FAs compared to the control grown without tadpoles. The concentration of deoxyanthocyanin in *A. pinnata* was shown to be only 15 % during summer when the plants were green compared to the winter time, when plants were red. In an experiment, both snail and tadpoles showed a clear preference for *A. filiculoides* over *A. pinnata*, while the latter one had a 20 times higher concentration of deoxyanthocyanins.
Therefore, antinutrients have a great impact on the nutritional quality of *Azolla*, while several environmental factors can have a great influence on the concentration of antinutrients. However, besides having antinutrional properties, dietary anthocyanins have

Figure 4.6: *Azolla filiculoides*, cold stressed in the middle tray and plants from inside the warmer polytunnel in the right tray, both from the same plant. Stressed *Azolla* turns visibly red.

also been shown to have beneficial effects on poultry, such as improved feed efficiency and pathogen resistance. Feeding trials have been conducted with certain feedstuff high in anthocyanins such as purple corn, Konini wheat and several fruit extracts [Changxing et al., 2018].
Fasakin (1999) showed that through processing *Azolla africana* biomass into a "leaf protein concentrate" through protein extraction, the amounts of cyanide, tannin and phytic acid could be lowered, while the protein content was more than doubled, making it more suitable as livestock feed. Cruz et al. (2011) found that *A. filiculoides* contained Trypsin inhibitors, phytates, soluble tannins and oxalates, which are all known antinutrients. Through lacto-fermentation, all antinutrients could be reduced substantially (except for phytates), as shown in table 4.3.

4.2.3 Feeding Trials

Similar to duckweed, *Azolla* is a cheap alternative to conventional protein feedstuff. Depending on the circumstances, it might even be cheaper than duckweed, as it has no requirements for nitrogen fertilizer. Becerra et al. (1995) states a price of fresh *Azolla* at 2.4 % of soybean meal in India.

Ruminants
Kumar (2008) reported about the effects of incorporating *Azolla sp.* into the diet of dairy buffaloes. All animals received 30 kg of Napier grass, 10 kg of paddy straw, 1 kg of rice bran and 1 kg of sesame oil meal. *Azolla* at 1.5 kg (fresh or dried not specified) per animal per day could replace 1 kg of sesame oil meal, while milk yield and milk fat content were slightly improved in comparison to the control.
Chatterjee et al. (2013) fed fresh *A. microphylla* to lactating crossbred cattle. All cattle received paddy straw, green fodder and concentrate mixture, while the experimental group received 2 kg of fresh *Azolla* per animal mixed into the ration. Milk yield and fat corrected milk yield increased by 11.2 and 12.5 % in the *Azolla* fed group, while total DM intake per fat corrected milk yield was reduced, indicating that the incorporation of *Azolla* into the ration improved the FCR.
In a second trial cattle received paddy straw (around 45 %), green fodder (around 15

%) and concentrate (around 40 %) as the control. The experimental group received 60 g of dried *Azolla* replacing 10 % of the concentrate. The experimental group had an increased growth rate of 9 % over the control and an improved FCR.
The authors suspect that certain trace elements, FAs, proanthocyanides or antioxidants are responsible for the improvement in animal performance and FCR.

Pigs
Becerra et al. (1990) substituted the protein supplement (soybean meal based) of growing-fattening pigs with 0, 15 and 30 % *A. filiculoides*. During the first phase of the trial, the experimental diets caused a reduction in weight gain, while in the second phase the pigs on the experimental diet gained weight faster than in the control. During the whole trial of 154 days, there was no difference in the growth rate of the pigs.

Broiler Chickens
Basak et al. (2002) found that broilers fed diets containing 5 % *A. pinnata* meal had better weight gain, FCR and higher overall profitability compared to the control or higher inclusion rates (10 and 15 %) of *Azolla*. Ara et al. (2015) found the best growth performance in broilers at 5 % *Azolla* meal inclusion in the diet compared to the control and higher inclusion rates (10, 15 and 20 %). The diet containing 5 % *Azolla* meal was also found to be the most profitable one.
Alalade and Iyayi (2006) fed Nera Brown chicks with diets containing 0, 5, 10 and 15 % *A. pinnata* meal. Both weight gain and feed efficiency was best at 10 % inclusion of *Azolla* meal.

Laying Hens
Boitai et al. (2018) found that *A. pinnata* meal can be included into the diet of laying hens at up to 10 % without adverse effects on laying performance and egg quality [Boitai et al., 2018].
Khatun et al. (2008) fed *A. pinnata* meal as part of the diet of laying hens at 0, 5, 10, 15 and 20 % inclusion. Laying performance decreased with increasing share of *Azolla* in the diet. However, egg production was most profitable at 20 % inclusion [Khatun et al., 2008].

Broiler Ducks
Acharya et al. (2015) fed fresh *Azolla pinnata* included at 0, 5 and 10 % DM basis into the diet of White Pekin broiler ducks. At 10 % the ducks showed the best performance, even though feed consumption was lowest, the weight gain was the highest of the 3 treatments. The inclusion of 10 % *Azolla* reduced the feed costs per live weight gain by over 15 %.
Becerra et al. (1995) used fresh *A. microphylla* as a replacement for boiled soybeans in the diet of meat ducks (Cherry Valley hybrids). The ducks received a fixed amount of soybeans calculated weekly according to their body weights and sugar cane juice *ad libitum*. The experimental groups had reductions of 15, 30, 45 and 60 % of their soybean ration and received sugar cane juice and *Azolla* both *ad libitum*. Weight gain was highest in the 15 % group, but declined with increasing replacement of soybeans with *Azolla*. The best profitability was achieved with 30 % reduction of soybeans.
In a second trial the ducks received the same amount of soybeans throughout the whole study period with sugar cane juice offered *ad libitum*. This time the experimental groups had reductions of 15, 25, 35 and 45 % of soybeans. Weight gain decreased with increasing

substitution of soybeans with *Azolla*. Even though the control group had the highest weight gain, the diet with 35 % reduction was the most profitable one.

Laying Ducks

Sujatha et al. (2013) fed two groups of layer ducks with commercial layer mash *ad libitum*, while the experimental group received supplementary fresh *A. pinnata* at 200 g per duck per day served separately from the mash. The consumption of concentrate feed in the experimental group was 30 % lower than in the control group. Laying performance remained almost unchanged between the two groups. Lawas et al. (1998) replaced half of the commercial concentrate ration of laying ducks with fresh *Azolla ad libitum*. Laying productivity and feed efficiency remained almost unchanged.

Swain et al. (2018) partially replaced the diet of White Pekin laying ducks with fresh *A. pinnata*. The control group received a standard concentrate only, the first experimental group received 10 % less concentrate and 100 g fresh *Azolla* per duck per day and the second experimental group received 20 % less concentrate and 200 g of fresh *Azolla*. Productivity parameters were best in the last group. Laying productivity increased by 46 % over the control, while FCR and egg weight was improved.

Aquaculture

As a fish feed, *Azolla* seems to be clearly inferior to duckweed. Filizadeh et al. (2004) investigated on the preference of grass carp (*Ctenopharyngodon idella*) for 10 different aquatic weeds, free floating and submerged. The most preferred plant was duckweed (*Lemna minor*), while *A. filiculoides* was only number 8.

Nekoubin and Sudagar (2012) fed 5 different diets to grass carp (*Ctenopharyngodon idella*) fingerlings (15.4 g) consisting of duckweed (*Lemna sp.*), *Azolla filiculoides*, alfalfa, pellets with 35 % protein and pellets with 25 % protein. The SGRs were at 0.55, 0.31, 1.18, 0.33 and 0.14 %/d, respectively. The FCRs were 35.29, 62.18, 15.61, 15.3 and 37.2. Unfortunately, the fish were fed only at 20 % for duckweed, *Azolla* and alfalfa and at 5 % for the pellets of their body weight 3 times per day. Alfalfa likely had a higher DM content than duckweed and *Azolla* and judging from the SGRs, the feeding rates were much below the requirements for optimal growth for all diets. As duckweed and *Azolla* have an approximately similar DM content, it can be concluded that duckweed has a more favourable effect on the growth of grass carp fingerlings than *Azolla*, as the reported weight gain was twice as high.

Fasakin et al. (2001) included dried *Azolla africana* into a commercial pelleted diet for Nile tilapia (*Oreochromis niloticus*) fingerlings to substitute the fish meal at 0, 5, 10, 20, 30 and 100 %. The SGR was 2.4, 2.1, 2.0, 1.8, 1.1, and 0.5, respectively. The FCR went from 1.6 (control) up to 7.5 for the 100 % *Azolla* for fishmeal substitution. The trial compared *Azolla* and duckweed inclusion into the diet at the same levels. Even though *Azolla* had a higher crude protein content than the duckweed (28.9 vs. 25.6 %), *Azolla* had a more negative impact on the weight of the Nile tilapia at every inclusion level compared to duckweed. The fish receiving 10 % *Azolla* grew less than the fish receiving 30 % duckweed.

Abou et al. (2007a) fed Nile tilapia (*Oreochromis nilotica*) fingerlings (15.5 g) with pellets containing 0, 10 and 20 % *Azolla filiculoides*. The SGRs were 2.35, 2.30 and 2.27 %/d, respectively. The diet containing 20 % *Azolla* was found to generate the highest profit.

Abou et al. (2007b) fed Nile tilapia (*Oreochromis niloticus*) fingerlings (16.2 g) with pellets containing 30, 35 or 40 % dried *Azolla filiculoides*. The SGR was 2.17, 2.14 and 2.11 %/d and the apparent FCR was 1.19, 1.22 and 1.23, respectively. Fiogbé et al.

(2004) fed pelleted diets to Nile tilapia (*Oreochromis nilotica*) fingerlings (1.7 g) containing 0, 15, 20, 30, 40 and 45 % *Azolla microphylla*. The pellets containing 15 % *Azolla* produced a higher weight gain in the fish than the control diet. *Azolla* incorporated at higher levels had a depressing effect on the SGR.
El-Sayed (1992) did experiments with both Nile tilapia (*Oreochromis niloticus*) fingerlings (2.54 g) and adults (40.33 g) fed with diets containing *Azolla pinnata* meal at different inclusion percentages from 0 to 100 %. With increasing inclusion of *Azolla*, SGR and FCR deteriorated. Adult fish on 100 % *Azolla* died. Utilization of *Azolla* was indicated to be higher in fingerlings than in adult Nile tilapia.
Almazan et al. (1986) fed Nile tilapia (*Oreochromis nilotica*) fingerlings with *Azolla pinnata ad libitum* in fresh form, powder and as pellets. The fingerlings were not able to even maintain their weight regardless of the preparation of *Azolla*.
Santiago et al. (1988) fed diets containing different levels of *Azolla pinnata* meal (8.50, 17.00, 25.46, 34.00 and 42.45 %) to Nile tilapia (*Oreochrimis niloticus*) fry (14.9 and 11.2 mg). Growth performance was best at a diet containing 34.00 and 42.45 % *Azolla* meal in two different experiments with varying ratios of diet ingredients besides *Azolla*. The diet containing no *Azolla* (control) produced the lowest weight gains in both experiments.
Majhi et al. (2006) grew grass carp (*Ctenopharyngodon idella*) fingerlings (23.5 g) in earthen ponds that were fertilized wth cow manure at 1 kg/m^2. One pond was left as a control, the other pond was additionally supplied with finely chopped fresh *A. caroliniana* at the rate of 10 % of the fish body weight once per day. The grass carp in the control had a SGR of 1.27 %/d, in the experimental treatment it was 1.65 %/d. The final average fish weight in the control group was 86.67 g, in the experimental group 270.34 g. It was concluded that *Azolla* was an ideal supplemental feed for the production of organic fish, as it could easily be produced locally using just cow manure and rock phosphate.

4.2.4 Integrated Systems

Azolla is traditionally used as a bio-fertilizer in rice cultivation. The flooded rice field is inoculated with *Azolla* by spreading 4.5 - 6 t/ha of fresh *Azolla* biomass 20 days before rice transplantation. After the whole water surface is covered with an *Azolla* mat, the field is drained and the *Azolla* ploughed into the soil. After that the field is filled up again and the rice seedlings are transplanted and fish such as Nile tilapia (*Oreochromis niloticus*) and/or certain carp species are introduced at 5,000 fingerlings per hectare. As the plowed in *Azolla* is rotting below the rice seedlings, nutrients are slowly released in the root zone. Some *Azolla* biomass remains floating, so it is growing in conjunction with the rice, contributing biomass and nitrogen to the system. Connected trenches and channels are integrated into the rice field to allow for enough water depth for the fish, occupying about 10 - 15 % of the space of the whole rice field. The fish mainly feed on the pond biology including *Azolla*, plankton and snails. They might also receive supplemental feed such as rice bran, broken rice, corn meal, groundnut cake and chicken or cattle manure [Hasan and Chakrabarti, 2009].
Cagauan and Nerona (1986) did field studies on the utilization of *Azolla* in rice-fish-cultivation. They used 3 different fertilizer regimes consisting of inorganic fertilizer (urea and ammonium phosphate), *Azolla* only and inorganic fertilizer (half dose) with *Azolla*, as portrayed in table 4.4. The combination of *Azolla* and inorganic fertilizer resulted in the highest yield of rice and fish. Also the utilization of *Azolla* allowed to reduce the inorganic fertilizer application by 50 %. However, the use of *Azolla* as a

biofertilizer requires more labour compared to inorganic fertilizer only.

Table 4.4: Use of *A. microphylla* as fertilizer in rice-fish culture system- fish species: *O. niloticus*, after Hasan (2009).

Initial weight (g)	**Fish density (Number/ ha)**	**Duration (days)**	**Fertilizer regimes**	**Fertilizer rate (kg/ha)**	**Quantity of N (kg/ha)**	**Fish yield (kg/ha)**	**Rice yield (kg/ha)**
8.9-9.4	5,000	75	*Azolla* only	3,750	5.63	45.1	2,567
			Inorganic fertilizer	150	38.5	45.0	3,096
			Azolla + Inorganic fertilizer	3,750 75	5.6 19.3	79.0	3,524

Cagauan and Pullin (1991) compared fish-rice farming systems stocked with either a Nile tilapia monoculture or a Nile tilapia, common carp and grass carp polyculture with and without *Azolla*. The fish yield for the monoculture was 0.63 t/ha and for the polyculture 0.7 t/ha without *Azolla*. With *Azolla* the yields were 1.20 and 1.06 t/ha. *Azolla*, also called mosquito fern, is known to inhibit the propagation of mosquitoes spending their larval stage in water bodies. Several diseases such as malaria are spread by mosquitoes, while the construction of open water bodies such as rice fields can substantially increase their numbers, posing a health risk to the local population.
Mwingira et al. (2009) found that mosquito productivity was low when the ponds were covered with *Azolla*. The higher the degree of coverage of the *Azolla* mat on the pond surface, the less mosquitoes could successfully breed and emerge.
Cheng et al. (2015) was investigating on the effects of different integrated rice systems on the rice yield and weed suppression. The systems were rice only (control), rice and *Azolla filiculoides*, rice and loaches (*Misgurnus anguillicaudatus*) a fish traditionally grown in rice paddy fields and a combination of rice, *Azolla* and loaches. *Azolla* significantly suppressed the weeds, the loaches even more so and in combination they eradicated them completely. Grain yields for the different treatments realtive to the control (100 %) were 137 % for rice with *Azolla*, 173 % for rice with the loaches and 229 % for the combination of rice, *Azolla* and loaches. The loaches usually feed on the pond biology only and are a popular food fish in South East Asia.
Diara and van Hove (1984) found that a mat of *Azolla* could reduce surface water loss by transpiration by more than 20 % under climatic conditions giving rise to a yearly transpiration loss of 1,343 mm. The authors pointed out the implications of growing paddy rice together with *Azolla* in order to save irrigation water.
Milicia and Favilli (1992) examined the use of *Azolla* as green manure as an alternative to synthetic nitrogen fertilizer for tomatoes. They found that *Azolla* biomass incorporated into the soil before seedling transplantation could fully replace ammonium sulphate fertilizer at equal nitrogen application rates. Ammonium sulphate at 100 kg N/ha increased the yield slightly more than *Azolla* at 100 kg N/ha, but at 200 kg N/ha, ammonium sulphate had a marginally lower yield increase than *Azolla* at 200 kg N/ha. A mixture of both gave the best results. The use of *Azolla* green manure accelerated the ripening of the fruits by 10 days and decreased damaged fruits by 8 % over synthetic fertilizer.

4.3 Usage of Floating Plants

Both duckweed and *Azolla* offer unique properties that can be exploited to solve many of today's problems concerning global food security and environmental pollution.

4.3.1 Soil Degradation

Global soil degradation is an imminent threat to food security. Both duckweed and *Azolla* have much higher growth rates and exhibit higher protein productivities than conventional crops, as shown in figure 4.7. The cultivation of floating plants can alleviate the pressure on arable land by reducing the area needed to produce conventional feed crops.

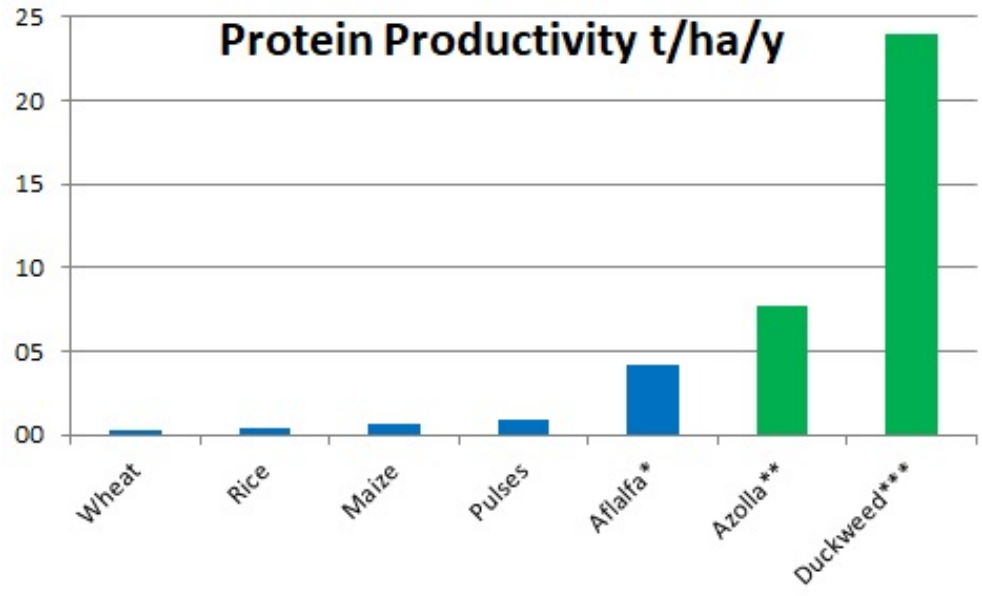

Figure 4.7: Protein productivity of major feed crops compared to *Azolla* and duckweed, after Clark and Tilman (2017), Frame (2005)*, Brouwer et al. (2017)** and Mohedano et al. (2012)***.

Duckweed and *Azolla* can provide a substantial amount of feed protein in livestock production. Both plants can be dried and incorporated into conventional feed preparations substituting common plant protein crops. The inclusion percentage has to be carefully considered for each livestock species and system, as there is a sweet spot where the reduction in feed cost and the reduction in growth rate determine maximum profitability as shown in figure 4.4.
Low inclusion rates of just 5 to 10 % already decrease the cost of feeding and in most cases will have a positive impact on the animal productivity. Increasing the inclusion rate above the threshold where livestock productivity begins to suffer might also be considered, as the reduction in feed cost can outweigh the reduction in productivity, resulting in a net gain in profitability. As the feeding costs are reduced with increasing inclusion of floating plants, livestock that can rely 100 % on floating plants might hold a high potential for maximized profitability, while minimizing resource consumption and dependence on import.
Skillicorn et al. (1993) showed that a duckweed based carp polyculture can produce a fish yield of 7.7 t/ha/y including the area needed for feed production (see calculation

in section 4.1.4). Assuming a dressing percentage of the fresh fish of 55 % (with 45 % waste) and a protein content of the remaining flesh of 16 %, this is equal to a protein productivity of 0.68 t/ha/y, which is superior to other livestock production systems, as shown in figure 4.8.

Floating plants can be grown in lined ponds that can be constructed on almost any

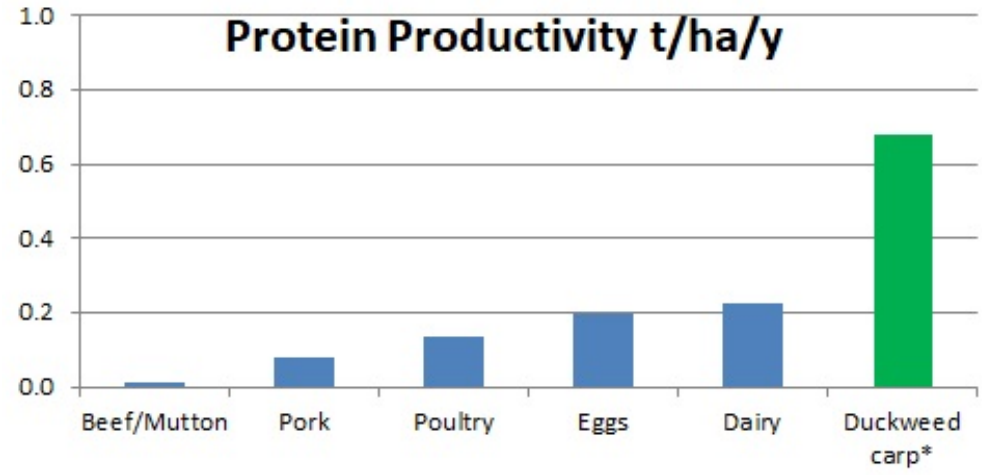

Figure 4.8: Protein productivity of conventional livestock systems compared to duckweed-based carp polyculture system, after Clark and Tilman (2017) and Skillicorn et al. (1993)*.

surface, earthen ponds can be established on degraded soils unfit for conventional crop production, so that competition with arable land is kept to a minimum.

Soil salinity is increasing globally and aggravated by irrigation of arable land with salty ground water. Half of the global irrigated fields and 20 % of all cultivated land is affected by salinity with negative impacts on crop yields [Zhu, 2001]. Skillicorn et al. (1993) described that duckweed can be cultivated on almost all soils that hold water well, except for alkaline soils. Water-logged soils and also salinized soils work well as duckweed tolerates up to 4,000 mg/l of salt, making duckweed a crop that can grow well where most other crops fail.

To summarize, the cultivation of duckweed and *Azolla* can alleviate pressure on arable land resources, as substantially more protein can be produced per area compared to conventional feed crops such as soybeans and the cultivation can take place on areas unfit for conventional crop production.

4.3.2 Nitrogen Dynamics

The supply of reactive nitrogen in the form of animal manure can easily surpass the demand in regions with a high density of livestock production facilities. Crops with a high nitrogen demand such as corn are often grown to assimilate as much of the nitrogen as possible, as transport or fees for disposal for liquid manure at sewage treatment plants are costly.

According to the data of Liang and MacKenzie (1994) one harvest of high yielding corn with a grain yield of 11.9 t/ha contains 245 kg of nitrogen in the total biomass. Assuming two harvests per year, corn plants can take up 490 kg N/ha/y. Mohedano et al. (2012) could show that duckweed can produce 68 t DM/ha/y of biomass containing 35 % crude protein grown in an open pond system for 1 year. This equals an uptake of nitrogen of

3,808 kg/ha/y. Therefore, duckweed can take up about 7 times more nitrogen per year than two harvests of corn, making it more suitable for regions with a surplus of manure. An added benefit is the fact that there is no water pollution during the cultivation of duckweed, provided the pond doesn't leak or flows over. Liang and McKenzie (1994) found the fertilizer nitrogen recovery in corn ranged from 9 to 58 % under different application rates and soils types. The remaining nitrogen either remained in the soil or was lost from the system.

While water pollution can be ruled out for the most part for duckweed cultivation, there can be a considerable loss of nitrogen through microbial nitrification and denitrification and also through volatilization of ammonia. Mohedano et al. (2012) found the loss of nitrogen in a duckweed pond through nitrification and denitrification to be at 72 % of total nitrogen removal, while the other 28 % were taken up by the duckweed. In the second pond with a lower nutrient load, the loss was only at 4 % and duckweed took up 96 % of all the nitrogen supplied.

As the use of synthetic nitrogen fertilizer is partially restricted in organic agriculture, the cultivation of *Azolla* can be especially useful both as an organic biofertilizer and as supplemental livestock feed. With a biological nitrogen fixation potential of 1,200 kg N/ha/y, *Azolla spp.* is the by far the most efficient biological alternative to synthetic nitrogen fertilizer production via the Haber Bosch process [Brouwer et al., 2017], as represented in figure 4.9.

Azolla can not only be used as a biofertilizer in conjunction with aquatic crops, such as paddy rice, but possibly with most crops. Milicia and Favili (1992) showed that *Azolla* could fully replace synthetic nitrogen fertilizer for tomatoes at 100 - 200 kg N/ha. Theoretically, 1 hectare of *Azolla* could supply 6 - 12 hectares of tomatoes as a nitrogen fertilizer, provided that *Azolla* is harvested frequently and digged into the soil.

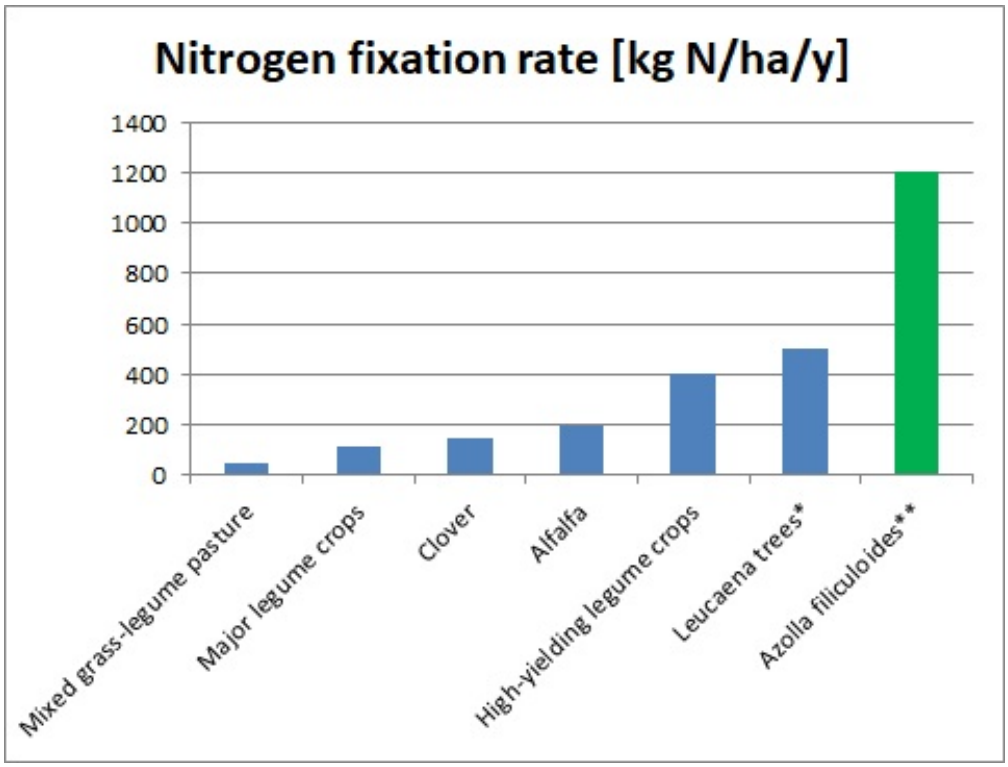

Figure 4.9: Nitrogen fixation rates of selected nitrogen fixing crops, after Herridge et al. (2008), Youkhana and Idol (2008)* and Brouwer et al. (2017)**.

4.3.3 Rebalancing the ω-6/ω-3 Ratio in Animal Products

The consequences of conventional feed crops on the FA profile and in particular on the ω-6/ω-3 ratio in animal products was already discussed in section 3.5. The increasing demand for products such as grass fed beef or "ω-3-eggs" are the result of an increasing awareness of the health-damaging effects of modern nutrition very often deficient in ω-3 FAs.
Floating plants have a great potential to increase the quality of animal products by lowering the ω-6/ω-3 ratio in possibly all domestic animals. The ratios in both *Azolla* and duckweed are below 1:1, unlike the ratio in common feed formulations that are based on grains and oil press cakes high in ω-6 FAs and very low in ω-3 FAs, as depicted in table 4.5. Due to the high protein content in floating plants, they can be substituted for protein feed products made from soybeans or sunflower seeds for instance, resulting in a more favourable ratio in the final product.

Table 4.5: Content of ω-6 and ω-3 FAs and the corresponding ratios in some seeds, forage plants and duckweed and *Azolla*, after U.S. Department of Agriculture (2008), Dierking et al. (2010)[a], Appenroth et al. (2017)[b] and Bhaskaran and Kannapan (2015)[c].

	ω-6 FAs [g/kg DM]	**ω-3 FAs [g/kg DM]**	**ω-6/ω-3 ratio**
Corn, yellow	18.9	0.6	32.3
Wheat, durum	8.3	0.4	19.4
Rice, brown, medium-grain, raw	8.0	0.4	22.4
Soybeans, mature seeds, raw	90.8	12.2	7.5
Sunflower seed kernels	214.3	0.7	311.5
Flaxseed	56.3	217.4	0.3
Orchardgrass[a]	3.98	26.7	0.1
Tall fescue[a]	3.56	28.4	0.1
Perennial ryegrass[a]	3.89	31.87	0.1
Alfalfa[a]	6.22	24.79	0.3
Duckweed (*Lemna minor*)[b]	9.28	20.42	0.5
Azolla filiculoides[c]	12.94	23.7	0.5

4.4 Comparison to Terrestrial Protein Crops

The culture of floating plants is obviously quite different from conventional crop production. The initial preparation of the land requires higher investment to form the pond area and to seal the ground. Harvesting is continuous throughout the year, every other day, while seed crops like soy are harvested once at the end of the season or alfalfa hay every few weeks during the growing season.
The water requirement for floating plants is substantially higher per area compared to terrestrial crops, but it is in fact lower relative to the production of biomass or protein (see table 4.6).
For the nitrogen requirements duckweed and *Azolla* are two extremes, duckweed can take up more nitrogen than any other crop and *Azolla* has no (reactive) nitrogen requirement at all.

Table 4.6: Comparison of duckweed, *Azolla*, soybeans and alfalfa in terms of water and nitrogen requirements, after Mohedano (2012)[a], Brouwer (2017)[b], FAO (n.d.)[c] and Frame (2005)[d]. Water requirement values for the floating plants[e] were estimated based on 5.4 mm daily water evapotranspiration per day for duckweed and 4.8 mm for *Azolla*. Water loss through harvested biomass was also accounted for .

	Duckweed	***Azolla***	**Soybeans**	**Alfalfa**
Harvest Biomass Yield [t/ha/y]	68.8[a]	35.5[b]	2.5[c]	17.8[d]
Protein Yield [t DM/ha/y]	24.0[a]	7.7[b]	1.0[c]	4.1[d]
Growing Season [d]	365[a]	365[b]	115[c]	365[d]
Water Requirement per Season [m^3/ha]	21,017[e]	18,195[e]	5,750[c]	12,460[d]
Water Requirement per Protein Produced [m^3/t]	880	2,360	5,750	3,039
Nitrogen Requirement per Protein Produced [kg/t]	411[a]	0[b]	15[b]	9[d]

4.5 Conclusion

Both duckweed and *Azolla* have been cultivated for hundreds of years in traditional integrated farming systems in South-East Asia. Both have the capacity to replace conventional protein feed sources in animal husbandry for all common livestock species, such as soybean meal to a great extent or even fully without compromising livestock performance. Their rate of productivity is among the highest of all plants, outperforming all major conventional crops in terms of biomass and protein production per area.
However, due to their very high water content, energy intensive drying is necessary for transport, processing or storage. While the fact that floating plants are grown in ponds is an advantage as it clearly reduces the competition for arable land, it also comes with obstacles: Both the cultivation and harvesting is quite labour intensive and very different to soil based crop production, see figure 4.10. Sophisticated mechanical equipment for large scale production does not exist yet.
Duckweed is among the best crops to maximise protein production, while minimizing environmental pollution, especially soil degradation and groundwater nitrate pollution.

Overall, *Azolla* is less productive and less favourable as an animal feed than duckweed is, but has enormous potential in organic agriculture due to its biological nitrogen fixation capacity that is far superior to all other nitrogen fixing plants known to date. This can be exploited in integrated systems for both crop fertilization and animal feed production without the use of synthetic nitrogen fertilizer.
Floating plants can not only replace conventional protein feed, but are a good source of pigments, vitamins, minerals and polyunsaturated FAs, especially the ω-3 FA ALA. Thereby they can contribute to the health of livestock and nutritional quality of animal products.
The next chapter depicts the practical research that was conducted involving floating plants as feed for poultry and fish.

Figure 4.10: Harvesting of mixed floating plants with a net.

5. Practical Research

The following experiments were all conducted on Biohof Moorriem in Elsfleth, Germany. The floating plants were grown in a 100 m^2 pond inside a polytunnel and a 120 m^2 outside pond. The duckweed, which was a mixture of several species was obtained locally, from the drainage ditches, wher it was naturally growing. *Azolla (A. filiculoides)* was obtained from a Botanical Garden.
The trials involving ducks and chickens were done in a consecutive order (Experiment 1 - 6) starting from 11.07. to 01.11.2019, while the experiments with the fish and the floating plants themselves (Experiment 7 and 8) were conducted parallel.

5.1 Experiment 1

The aim of this trial was to investigate the effects of offering floating plants to laying ducks *ad libitum* in addition to their regular concentrate feed on feed consumption, laying performance and egg nutritional quality parameters.

5.1.1 Materials and Methods

For this trial 30 ducks at the age of 11 months of the variety "Deutsche Campbellente" were randomly assigned into two groups. Both groups contained 2 drakes and 13 layer ducks and were fed a 1:1 (FW basis) mixture of wheat grain and supplementary concentrate *ad libitum*. Both groups had free access to 500 m^2 of pasture and 60 m^2 of open pond area each, of the same pond divided into two partitions. The control group had no plants in their pond partition, the partition of the experimental group had both *Azolla filiculoides* and mixed species of duckweed (*Spirodela polyrrhiza, Lemna gibba* and *Wolffia arrhiza*) growing in it (figure 5.1).

Figure 5.1: Ducks of the experimental group swimming in the pond with floating plants.

The nutritional parameters of the concentrate and the floating plants according to the analysis is depicted in table 5.1 and the composition of the diet in table 5.2. The trial lasted 42 days, the ducks were weighed on day 0 and day 42 and eggs were collected daily, counted and weighed for each group. At the end of the trial, 6 eggs were collected of each group and sent in for analysis of nutritional parameters and FA profiles (one mixed sample out of six eggs for each group). Eggs were also analysed for contamination with Salmonella. All analyses were done at the Institute of Food Quality of LUFA Nord-West. The original analysis results can be found in the Appendices (Appendix B, figures B.1 to B.11 for feed components, Appendix C, figures C.1 to C.26 for egg analysis)

Table 5.1: Nutritional parameters of the feed components in experiment 1

	Dry matter [%]	Crude protein [%]	Starch [%]	Sugar [%]	Fat [%]	Ash [%]	ω-6 FAs [%]	ω-3 FAs [%]	ω-6/ ω-3 ratio
Wheat	86.10	12.43	66.67	2.67	2.90	1.86	1.61	0.10	16.29
Suppl. conc.	92.40	31.39	4.00	4.44	9.74	20.13	2.47	0.23	10.58
Azolla	5.00	29.30	5.80	6.20	6.10	14.30	0.82	1.34	0.62
Duckweed	5.60	33.70	12.40	5.40	9.30	16.30	1.83	2.90	0.63

Table 5.2: Diet composition of the concentrate in experiment 1 for both control and experimental group. Experimental group had access to floating plants *ad libitum*.

Ingredients	
Wheat grain [%] of DM	48.24
Supplementary concentrate [%] of DM	51.76
Nutrient composition (calculated)	
DM [%] of FW	89.25
Crude protein [%] of DM	22.24
Starch [%] of DM	34.23
Sugar [%] of DM	3.59
Fat [%] of DM	6.44
Ash [%] of DM	11.32
ω-6 FAs [%] of DM	2.06
ω-3 FAs [%] of DM	0.17
ω-6/ ω-3 ratio	12.20

The supplementary concentrate was mainly composed of oilseed press cake from soy and sunflower with added vitamins, minerals and trace elements a detailed list of ingredients is given in the Appendix B (figure B.4).

5.1.2 Results

The effect of the two different feeding regimes on performance parameters are shown in table 5.3. Table 5.4 gives the nutritional composition of the eggs from the control and experimental group.

Table 5.3: Performance parameters in experiment 1 in control and experimental group

	Control group	**Experimental group**
Initial body weight [kg]	2.09±0.25	2.05±0.24
Final body weight [kg]	2.21±0.18	2.17±0.21
Feed consumption DM per duck per day [g/d]	171.98	168.87
Egg number [-]	206	203
Egg weight [g]	78.72±8.11	73.75±5.64
Egg mass laid per duck per day [g/d]	25.74	23.76
FCR (feed:egg)	6.68	7.11
Survivability [%]	86.67	93.33

Table 5.4: Nutritional parameters of the eggs in experiment 1 of the control and experimental group, according to analysis

	Control group	**Experimental group**
Vitamin A [mg/kg]	2.982	2.574
Vitamin E (Alpha-Tocopherol) [mg/kg]	44	46
Total fat [%]	10.1	10.9
Saturated FAs [%]	2.66	2.87
Monounsaturated FAs [%]	5.74	6.55
ω-6 FAs [%]	1.38	1.22
ω-3 FAs [%]	0.22	0.20
DHA [%]	0.05	0.04
ω-6/ω-3 ratio	6.27	6.10

5.1.3 Discussion and Conclusion

The offering of floating plants had only little effect on laying performance and egg quality. The feed consumption of concentrate was just 2 % lower in the experimental group (table 5.3).
Sujatha et al. (2013) found that laying ducks that were offered fresh *Azolla* were consuming 30 % less concentrate.
However, the fact that the concentrate had a ω-6/ω-3 ratio of 12.2 and the eggs were measured at 6.27 and 6.10 for the control group and the experimental group, respectively (table 5.4) is suggesting that the ducks in both groups received a substantial part of their diet through scavenging. It might be possible that both groups were able to feed on phyto- or zooplankton growing in the pond, as the ducks in both groups have been observed to spend the majority of their time in or right next to the pond.
This trial had serious weaknesses. During the trial one laying duck was killed by the drakes and two other laying ducks disappeared, as they were probably taken by a predator. Close to the end of the trial, a magpie was observed as it picked up a fresh duck egg and flew away with it. The amount of eggs that got taken away like this is unclear. Consequently, the validity of this trial is very limited.

As a conclusion, the ducks had little interest in the floating plants as they had free

access to concentrate feed and apparently also found ample amounts of nutrition in their surrounding area. Therefore, ducks should be restricted in their concentrate feed in order to increase their interest in the floating plants. For the following trials, the experimental groups received less supplementary concentrate to encourage consumption of the floating plants. Additionally, a protected enclosure was built to protect the birds.

5.2 Experiment 2

As the first trial showed very little effects, in this trial the diet of the experimental group was restricted and fresh *Azolla* was offered daily to the experimental group.

5.2.1 Materials and Methods

Twenty-six ducks (Deutsche Campbellente) were randomly assigned into two groups, each group containing two drakes and 11 laying ducks. They were kept on a fenced in pasture with an area of 90 m^2 for each group, from this trial on the whole enclosure was protected from the crows with a net and an electrical fence to keep predators out. There was no access to the pond anymore, to enable better control of feed intake. The trial lasted for 19 days.
Both groups received concentrate feed *ad libitum*, however the control group had still a 1:1 mixture of wheat and supplementary concentrate, while the experimental group had a 10:7 mixture. Both groups had a 90 l rubber tub in their enclosure that was drained and refilled with water every day to provide drinking water and so that the ducks were able to wash their heads, as they could no longer access the pond. The experimental group received fresh *Azolla ad libitum* that was placed in the rubber tub every day so it was floating on the water until it was eaten up. Left over *Azolla* was removed every morning and weighed to calculate the total amount that was eaten by the ducks. The consumed concentrate was also weighed. Eggs were collected daily, counted and weighed. The ducks were weighed at the beginning and end of the trial. The nutritional analysis of the concentrate and *Azolla* is depicted in table 5.5 and the composition of the diet of both groups in table 5.6. At the end of the trial, 6 eggs were collected of each group and sent in for analysis of nutritional parameters and FA profiles (one mixed sample out of six eggs for each group). All analyses were done at the Institute of Food Quality of LUFA Nord-West.

Table 5.5: Nutritional parameters of the feed components in experiment 2

	Dry matter [%]	Crude protein [%]	Starch [%]	Sugar [%]	Fat [%]	Ash [%]	ω-6 FAs [%]	ω-3 FAs [%]	ω-6/ ω-3 ratio
Wheat	86.10	12.43	66.67	2.67	2.90	1.86	1.61	0.10	16.29
Suppl. conc.	92.40	31.39	4.00	4.44	9.74	20.13	2.47	0.23	10.58
Azolla	5.00	29.30	5.80	6.20	6.10	14.30	0.82	1.34	0.62

Table 5.6: Diet composition in experiment 2 of the control and experimental group.

Ingredients	**Control group**	**Experimental group**
Wheat grain [%] of DM	48.24	50.50
Supplementary concentrate [%] of DM	51.76	38.00
Azolla [%] of DM	-	11.40
Nutrient composition (calculated)		
DM [%] of FW	89.25	30.49
Crude protein [%] of DM	22.24	21.56
Starch [%] of DM	34.23	35.92
Sugar [%] of DM	3.59	3.74
Fat [%] of DM	6.44	5.87
Ash [%] of DM	11.32	10.22
ω-6 FAs [%] of DM	2.06	1.85
ω-3 FAs [%] of DM	0.17	0.29
ω-6/ ω-3 ratio	12.20	6.35

5.2.2 Results

The effect of the two different feeding regimes on performance parameters are shown in table 5.7. Table 5.8 gives the composition of the eggs from the control and experimental group.

Table 5.7: Performance parameters in experiment 2 in control and experimental group

	Control group	**Experimental group**
Initial body weight [kg]	2.13±0.17	2.15±0.17
Final body weight [kg]	2.26±0.19	2.15±0.13
Feed consumption DM per duck per day [g/d]	158.12	133.26
Egg number [-]	79	60
Egg weight [g]	75.14±6.81	76.65±5.87
Egg mass laid per duck per day [g/d]	24.03	18.62
FCR (feed:egg)	6.58	7.16
Survivability [%]	100	100

Table 5.8: Nutritional parameters of the eggs in experiment 2 of the control and experimental group, according to analysis

	Control group	**Experimental group**
Vitamin A [mg/kg]	3.210	3.390
Vitamin E (Alpha-Tocopherol) [mg/kg]	49	39
Total fat [%]	14.4	15.6
Saturated FAs [%]	4.39	4.8
Monounsaturated FAs [%]	7.68	8.5
ω-6 FAs [%]	1.96	1.89
ω-3 FAs [%]	0.22	0.23
DHA [%]	0.04	0.05
ω-6/ω-3 ratio	8.91	8.22

5.2.3 Discussion and Conclusion

In this trial, judging from the concentrate feed consumption, ducks of the experimental group ate considerably more *Azolla* than in the first trial due to the lower percentage of supplementary concentrate in their feed. Concentrate feed consumption was clearly depressed in the experimental group, 88.4 % of the wheat and 61.9 % of supplementary concentrate were eaten relative to the control group. Also the amount of total feed consumed was 15.7 % less on a DM basis in the experimental group (5.7).
While the ducks in the control group were gaining body weight during the trial, in the experimental group body weights stagnated on average. Laying productivity and feed conversion were reduced in the experimental group (table 5.7).
As shown in table 5.8 *Azolla* in the diet apparently increased Vitamin A and fat content and decreased Vitamin E content. ω-6 FAs were slightly decreased and ω-3 FAs increased, so that the ω-6/ω-3 ratio was improved from 8.91 to 8.22.

Azolla made up 11.4 % of the duck's diet on a DM basis. On average one duck consumed 304 g of fresh *Azolla* per day given free choice. Sujatha et al. (2013) observed that local laying ducks fed 200 g fresh *Azolla* per duck per day ate 153 g of concentrate. Hence, *Azolla* was making up 8.0 % of the diet on a DM basis. The control group that did not receive *Azolla* ate 219 g of concentrate, so that 13.2 g of *Azolla* were replacing 66 g of concentrate in the experimental group without affecting laying performance. Apparently, *Azolla* must have had a profound effect on the feed utilization efficiency in the ducks, as the experimental group laid more eggs with less total feed consumed in comparison to the control group.
Swain et al. (2018) observed that White Pekin laying ducks fed 200 g of fresh *Azolla* per day, consumed less feed and had significantly higher laying productivity.
Acharya et al. (2015) saw the highest weight gain and lowest FCR in White Pekin broiler ducks at 10 % inclusion of *Azolla* meal.
Lawas et al. (1998) reported that half of the commercial feed ration of Philippine Mallard layer ducks could be replaced with *Azolla* without any detrimental effects on performance.
As a conclusion, the offering of *Azolla* reduced the consumption of concentrate feed substantially, however, laying performance was observed to be decreased. The positive effect on the ω-6/ω-3 ratio in the eggs was rather weak. The positive effects on laying performance and feed conversion efficiency reported in the cited literature stand in contrast to the findings of this trial. Reasons for this discrepancy might be the short duration of

this trial, genetic differences in the duck strain or the quality of the used *Azolla*.

5.3 Experiment 3

The previous trial was repeated with duckweed instead of *Azolla*.

5.3.1 Materials and Methods

The previous trial with ducks was replicated, only this time duckweed was offered instead of *Azolla* in the experimental group. The duckweed consisted of a mixture of *Spirodela polyrrhiza, Lemna gibba* and *Wolffia arrhiza.*The trial lasted for 16 days. The nutritional analysis of the concentrate and duckweed is depicted in table 5.9 and the composition of the diet of both groups in table 5.10.

Table 5.9: Nutritional parameters in experiment 3 of the feed components

	Dry matter [%]	Crude protein [%]	Starch [%]	Sugar [%]	Fat [%]	Ash [%]	ω-6 FAs [%]	ω-3 FAs [%]	ω-6/ ω-3 ratio
Wheat	86.10	12.43	66.67	2.67	2.90	1.86	1.61	0.10	16.29
Suppl. conc.	92.40	31.39	4.00	4.44	9.74	20.13	2.47	0.23	10.58
Duckweed	5.60	33.70	12.40	5.40	9.30	16.30	1.83	2.90	0.63

Table 5.10: Diet composition in experiment 3 of the control and experimental group.

Ingredients	**Control group**	**Experimental group**
Wheat grain [%] of DM	48.24	46.85
Supplementary concentrate [%] of DM	51.76	35.20
Duckweed [%] of DM	-	17.95
Nutrient composition (calculated)		
DM [%] of FW	89.25	24.21
Crude protein [%] of DM	22.24	22.92
Starch [%] of DM	34.23	34.87
Sugar [%] of DM	3.59	3.78
Fat [%] of DM	6.44	6.46
Ash [%] of DM	11.32	10.88
ω-6 FAs [%] of DM	2.06	1.95
ω-3 FAs [%] of DM	0.17	0.65
ω-6/ ω-3 ratio	12.20	3.01

5.3.2 Results

The effect of the two different feeding regimes on performance parameters are shown in table 5.11. Table 5.12 gives the composition of the eggs from the control and experimental group.

Table 5.11: Performance parameters in experiment 3 in control and experimental group

	Control group	**Experimental group**
Initial body weight [kg]	2.26±0.19	2.15±0.13
Final body weight [kg]	2.37±0.22	2.28±0.14
Feed consumption DM per duck per day [g/d]	117.05	110.83
Egg number [-]	40	40
Egg weight [g]	75.10±7.56	78.10±7.00
Egg mass laid per duck per day [g/d]	14.44	15.02
FCR (feed:egg)	8.1	7.38
Survivability [%]	100	100

Table 5.12: Nutritional parameters of the eggs in experiment 3 of the control and experimental group, according to analysis

	Control group	**Experimental group**
Vitamin A [mg/kg]	2.652	3.000
Vitamin E (Alpha-Tocopherol) [mg/kg]	39	47
Total fat [%]	12.3	15.1
Saturated FAs [%]	4.00	4.85
Monounsaturated FAs [%]	6.42	8.27
ω-6 FAs [%]	1.59	1.49
ω-3 FAs [%]	0.16	0.29
DHA [%]	0.04	0.06
ω-6/ω-3 ratio	9.94	5.14

5.3.3 Discussion and Conclusion

The offering of duckweed had profound effects on all examined parameters and was much more beneficial compared to *Azolla* in the previous trial.

The ducks in the experimental group consumed 92.0 % of wheat and 64.4 % supplementary concentrate of what the control group ate. On average, duckweed was eaten at 355 g per duck per day, constituting 17.95 % of the diet on a DM basis. As depicted in table 5.11, both groups were gaining body weight during the trial, but the control group gained relatively more weight. The laying performance was the same for both groups in egg count, but total egg mass in the experimental group was higher. Apparently, FCR was improved by the offering of fresh duckweed.
Men et al. (2002) fed fresh duckweed *ad libitum* to local breeding ducks and Cherry Valley breeding ducks with concentrate containing differing amounts of fishmeal and soybean meal also being offered *ad libitum*. Depending on the composition of the concentrate feed, local breeding ducks consumed between 156 to 167 g of concentrate and 701 to 722 g fresh duckweed per day. Cherry Valley ducks consumed between 189 to 204 g concentrate and 806 to 834 g fresh duckweed. Laying rate was observed to decline with reduced percentage of soybeanmeal and fishmeal in the diet.
Khandaker et al. (2007) included duckweed meal at 0, 5, 10 and 15 % into the diet of

Jinding layer ducks replacing mustard oil cake. With increasing amounts of duckweed in the diet laying performance as well as FCR declined.
Khanum et al. (2005) found that restricting the daily concentrate ration for growing ducks by half and offering them fresh duckweed reduced the daily weight gain by 28 %. Ducks that received no concentrate, but only access to a pond with duckweed died within 3 weeks.
Egg quality was markedly improved in the experimental group as seen in table 5.12. Vitamin A and E were higher, total fat content was higher and the ω-6/ω-3 ratio changed from 9.94 to 5.14. Also the DHA content per egg was increased by a third.
While the ω-6/ω-3 ratio was lower in the eggs than in the diet of the control group, it was the other way around in the experimental group.
Duck eggs collected from the wild from the 5 different duck species king eider (*Somateria spectabilis*), the lesser scaup (*Aythya affinis*), the mallard (*Anas platyrhynchos*), the green-winged teal (*Anas crecca*) and the gadwall (*Anas streperi*) were analysed regarding their FA profile. The ω-6/ω-3 ratio (calculated from triacylglycerols, phospholipids and cholesteryl esters) ranged from 0.86 to 2.9. The DHA content ranged from 5.9 to 7.0 % [Speake et al., 2002].
Compared to the eggs produced in this trial, eggs from wild ducks still had a more favourable ω-6/ω-3 ratio and about 10 or more times as much DHA. However, the eggs from the experimental group came closer to the wild duck eggs than the ones from the control group, judging from the FA composition.
The content of the long-chain ω-3 FAs can be increased in eggs by the incorporation of fish oil into the poultry diet. It was shown that the addition of 2 to 6 % cod liver oil increased the EPA content in duck eggs from 0.04 % (control) to 0.25 to 0.84 % and the DHA content from 0.51 (control) to 2.72 to 5.7 %. However, with increasing amounts of fish oil in the diet of Tsaiya laying ducks, the eggs developed a fishy taste [Chen and Hsu, 2003].
As a conclusion, duckweed was more palatable to ducks than *Azolla*, had a beneficial effect on the FCR and laying performance (same egg count, but bigger eggs), increased Vitamin A and E content in the eggs and led to a substantially better ω-6/ω-3 ratio compared to the control. The duckweed supplied 19.8 % of the total crude protein in the diet and is therefore a potential alternative for other common protein sources such as soybean meal.

5.4 Experiment 4

As the laying productivity of the ducks was declining, they were replaced with laying hens for further trials. The previous trial with duckweed was replicated using the chickens instead of ducks.

5.4.1 Materials and Methods

Thirty-two Auracana hens at the age of 38 weeks were randomly distributed into two groups. The chickens were kept in the same enclosure where the ducks were kept, after they were sold. This trial was conducted in a similar way as the previous trial with ducks receiving duckweed. The duckweed was harvested, drained and offered to the experimental group daily *ad libitum* in a trough, separate from the concentrate feed and also from the water supply, as depicted in figure 5.2. The trial was conducted for 17 days. The nutritional analysis of the concentrate and duckweed is depicted in table 5.13 and the composition of the diet of both groups in table 5.14.

Figure 5.2: Chickens in the experimental group being fed fresh duckweed in a trough.

Table 5.13: Nutritional parameters of the feed components in experiment 4

	Dry matter [%]	Crude protein [%]	Starch [%]	Sugar [%]	Fat [%]	Ash [%]	ω-6 FAs [%]	ω-3 FAs [%]	ω-6/ ω-3 ratio
Wheat	86.10	12.43	66.67	2.67	2.90	1.86	1.61	0.10	16.29
Suppl. conc.	92.40	31.39	4.00	4.44	9.74	20.13	2.47	0.23	10.58
Duckweed	5.60	33.70	12.40	5.40	9.30	16.30	1.83	2.90	0.63

Table 5.14: Diet composition in experiment 4 of the control and experimental group.

Ingredients	**Control group**	**Experimental group**
Wheat grain [%] of DM	48.24	47.28
Supplementary concentrate [%] of DM	51.76	35.53
Duckweed [%] of DM	-	17.20
Nutrient composition (calculated)		
DM [%] of FW	89.25	24.97
Crude protein [%] of DM	22.24	22.82
Starch [%] of DM	34.23	35.07
Sugar [%] of DM	3.59	3.77
Fat [%] of DM	6.44	6.43
Ash [%] of DM	11.32	10.83
ω-6 FAs [%] of DM	2.06	1.95
ω-3 FAs [%] of DM	0.17	0.63
ω-6/ ω-3 ratio	12.20	3.11

5.4.2 Results

The effect of the two different feeding regimes on performance parameters are shown in table 5.15. Table 5.16 gives the composition of the eggs from the control and experimental group.

Table 5.15: Performance parameters in experiment 4 in control and experimental group

	Control group	**Experimental group**
Initial body weight [kg]	1.68±0.13	1.78±0.11
Final body weight [kg]	1.62±0.14	1.74±0.11
Feed consumption DM per chicken per day [g/d]	74.48	96.48
Egg number [-]	84	96
Egg weight [g]	59.54±5.00	60.13±7.93
Egg mass laid per chicken per day [g/d]	18.39	21.22
FCR (feed:egg)	4.05	4.55
Survivability [%]	100	100

Table 5.16: Nutritional parameters of the eggs in experiment 4 of the control and experimental group, according to analysis

	Control group	**Experimental group**
Vitamin A [mg/kg]	3.000	2.790
Vitamin E (Alpha-Tocopherol) [mg/kg]	57	34
Total fat [%]	12.3	10.6
Saturated FAs [%]	4.43	3.95
Monounsaturated FAs [%]	6.01	5.11
ω-6 FAs [%]	1.57	1.26
ω-3 FAs [%]	0.15	0.15
DHA [%]	0.04	0.03
ω-6/ω-3 ratio	10.47	8.40

5.4.3 Discussion and Conclusion

The duckweed was well accepted by the hens in the experimental group and made up 17.20 % of the total diet (table 5.14), only slightly less than what was observed with the ducks (17.95 %). On average one hen ate 296 g of fresh duckweed per day. Hens fed duckweed consumed 26.69 % more wheat, but 11.10 % less supplementary concentrate. Both groups lost weight during the trial, but the control group slightly more (table 5.15). Feed consumption in the experimental group increased, laying performance increased and FCR increased.

Akter et al. (2011) fed different inclusion levels from 0 to 15 % duckweed (*Lemna minor*) to Star Cross Brown laying pullets. They concluded that duckweed can be fed up to 13 % without any harmful effects on productivity.

Chantiratikul et al. (2010) obtained the best results at an inclusion rate of 9.1 % duckweed (*Wolffia globosa*) in the diet of Rohman laying hens, replacing soybeanmeal by 75 %.

Anderson et al. (2011) fed a diet containing 12.6 % duckweed (*Spirodela polyrrhiza*) to white leghorn laying hens and observed improvements in laying performance and FCR.

Haustein et al. (1990) found that laying performance and FCR was improved in TOPAZ laying hens with 25 % duckweed (*Lemna gibba*) in their diet. At 40 % inclusion, performance was only slightly compromised. In HyLine Leghorn layers at 15 %, they observed

improved laying performance and FCR in week 2 and 6, but both parameters worsened in week 10 compared to the control.
The feeding of duckweed led to a reduction in vitamin A and E and total fat content in the eggs (table 5.16). The ω-6 FA content was reduced, while the ω-3 FA content remained unchanged, the ω-6/ω-3 ratio improved from 10.47 to 8.40. The DHA content remained the same per fat content.
Anderson et al. (2011) saw an increase in the ω-3 FA content from 0.09 to 0.15 % in the eggs when the hens were fed a diet containing 12.6 % duckweed meal. ω-6 FAs were not analysed.
In conclusion duckweed showed good potential as an alternative protein source for laying hens. The effects were increased feed intake, higher laying performance, an improved FA profile in the eggs, but also a higher FCR.

5.5 Experiment 5

The duckweed offered to the chickens in the previous trial was replaced with *Azolla* in this trial.

5.5.1 Materials and Methods

In this trial, fresh *Azolla filiculoides* was offered to the experimental group in addition to the concentrate feed. The trial lasted for 19 days. The nutritional analysis of the concentrate and duckweed is depicted in table 5.17 and the composition of the diet of both groups in 5.18.

Table 5.17: Nutritional parameters of the feed components in experiment 5

	Dry matter [%]	Crude protein [%]	Starch [%]	Sugar [%]	Fat [%]	Ash [%]	ω-6 FAs [%]	ω-3 FAs [%]	ω-6/ ω-3 ratio
Wheat	86.10	12.43	66.67	2.67	2.90	1.86	1.61	0.10	16.29
Suppl. conc.	92.40	31.39	4.00	4.44	9.74	20.13	2.47	0.23	10.58
Azolla	5.00	29.30	5.80	6.20	6.10	14.30	0.82	1.34	0.62

Table 5.18: Diet composition in experiment 5 of the control and experimental group.

Ingredients	**Control group**	**Experimental group**
Wheat grain [%] of DM	48.24	55.21
Supplementary concentrate [%] of DM	51.76	41.48
Azolla [%] of DM	-	3.31
Nutrient composition (calculated)		
DM [%] of FW	89.25	57.08
Crude protein [%] of DM	22.24	20.85
Starch [%] of DM	34.23	38.66
Sugar [%] of DM	3.59	3.52
Fat [%] of DM	6.44	5.85
Ash [%] of DM	11.32	9.85
ω-6 FAs [%] of DM	2.06	1.94
ω-3 FAs [%] of DM	0.17	0.20
ω-6/ ω-3 ratio	12.20	9.92

5.5.2 Results

The effect of the two different feeding regimes on performance parameters are shown in table 5.19. Table 5.20 gives the composition of the eggs from the control and experimental group.

Table 5.19: Performance parameters in experiment 5 in control and experimental group

	Control group	**Experimental group**
Initial body weight [kg]	1.62±0.14	1.74±0.11
Final body weight [kg]	1.78±0.14	1.73±0.12
Feed consumption DM per chicken per day [g/d]	107.31	110.52
Egg number [-]	53	91
Egg weight [g]	59.32±6.28	61.33±6.83
Egg mass laid per chicken per day [g/d]	10.34	18.36
FCR (feed:egg)	10.38	6.02
Survivability [%]	100	100

Table 5.20: Nutritional parameters of the eggs in experiment 5 of the control and experimental group, according to analysis

	Control group	**Experimental group**
Vitamin A [mg/kg]	2.730	2.460
Vitamin E (Alpha-Tocopherol) [mg/kg]	42	40
Total fat [%]	10.9	10.8
Saturated FAs [%]	3.77	3.76
Monounsaturated FAs [%]	5.35	5.71
ω-6 FAs [%]	1.52	1.11
ω-3 FAs [%]	0.12	0.11
DHA [%]	0.03	0.04
ω-6/ω-3 ratio	12.67	10.09

5.5.3 Discussion and Conclusion

The preference of both ducks and chickens of duckweed over *Azolla* was confirmed in this trial. The hens consumed 73 g of fresh *Azolla* per hen per day, making up just 3.31 % of the total diet (table 5.18). However, the offering of *Azolla* apparently substantially increased laying productivity much more so than duckweed in the previous trial and also improved the FCR (table 5.19), which was not observed with duckweed. The experimental group consumed 17.89 % more wheat and 17.48 % less supplementary concentrate. While the experimental group slightly lost body weight on average, the control group gained weight.
Boitai et al. (2018) found that the feed efficiency of Vanaraja laying hens was slightly improved with 5 % *Azolla* meal in the diet and slightly decreased with 10 %. Laying performance was almost not affected.
When Khatun et al. (2008) fed *Azolla* at 0, 5, 10, 15 and 20 % inclusion to laying hens, laying performance and FCR deteriorated with increasing share of *Azolla* in the diet.
The *Azolla* used in this trial was infested with aphids and *Azolla* weevils and was stressed, judging from its color that had turned from green to red. This was not the case when *Azolla* was fed to the ducks, which might explain the pronounced decrease in apparent palatability for the chickens in comparison to the ducks.
As Cohen et al. (2002) showed, *Azolla* can up-regulate its antinutrient content, when predators are feeding on it.
As shown in table 5.20 the eggs from the *Azolla* group had slightly less vitamin A, E and total fat. Even the ω-3 FA content was slightly lower than in the control, but ω-6 FAs were substantially reduced, so that the ω-6/ω-3 ratio dropped from 12.67 to 10.09. *Azolla* apparently had a more beneficial effect on most parameters of the egg than duckweed did, even though it made up a much smaller percentage of the diet.

5.6 Experiment 6

In this trial both groups of chickens received duckweed in pre-mixed diets that were supplemented with 5 and 10 % flax seed in order to see how the ω-6/ω-3 ratio in the diet translates into the egg at lower ω-6/ω-3 ratios near 1:1.

5.6.1 Materials and Methods

This trial was designed similar to the previous ones, but both groups T1 and T2 received prepared diets, where fresh duckweed was mixed together with the concentrate feed every day, as depicted in figure 5.3.

Figure 5.3: Concentrate together with fresh duckweed before (right) and after mixing (left).

The hens were fed once a day, both groups got the same amount every day. Left over feed from the previous day was weighed and discarded, before the hens received fresh feed. The T1 group received a diet with 5 % flaxseed grit and T2 got 10 %, partially replacing the supplementary concentrate. The trial lasted for 17 days. The nutritional analysis of the concentrate and duckweed is depicted in table 5.21 and the composition of the diet of both groups in table 5.22.

Table 5.21: Nutritional parameters of the feed components in experiment 6

	Dry matter [%]	Crude protein [%]	Starch [%]	Sugar [%]	Fat [%]	Ash [%]	ω-6 FAs [%]	ω-3 FAs [%]	ω-6/ ω-3 ratio
Wheat	86.10	12.43	66.67	2.67	2.90	1.86	1.61	0.10	16.29
Suppl. conc.	92.40	31.39	4.00	4.44	9.74	20.13	2.47	0.23	10.58
Flaxseed grit	91.40	23.41	2.52	1.20	46.72	3.06	6.63	23.36	0.28
Duckweed	5.60	33.70	12.40	5.40	9.30	16.30	1.83	2.90	0.63

Table 5.22: Diet composition in experiment 6 of the control and experimental group.

Ingredients	**Control group**	**Experimental group**
Wheat grain [%] of DM	45.67	45.67
Supplementary concentrate [%] of DM	29.33	24.33
Flaxseed grit	5.00	10.00
Duckweed [%] of DM	20.00	20.00
Nutrient composition (calculated)		
DM [%] of FW	67.81	67.72
Crude protein [%] of DM	22.79	22.39
Starch [%] of DM	34.22	34.15
Sugar [%] of DM	3.66	3.50
Fat [%] of DM	8.38	10.23
Ash [%] of DM	10.17	9.31
ω-6 FAs [%] of DM	2.16	2.37
ω-3 FAs [%] of DM	1.86	3.02
ω-6/ ω-3 ratio	1.16	0.78

5.6.2 Results

The effect of the two different feeding regimes on performance parameters are shown in table 5.23. Table 5.24 gives the composition of the eggs from the control and experimental group.

Table 5.23: Performance parameters in experiment 6 in control and experimental group

	Control group	**Experimental group**
Initial body weight [kg]	1.78±0.14	1.73±0.12
Final body weight [kg]	1.63±0.10	1.59±0.13
Feed consumption DM per chicken per day [g/d]	121.52	121.86
Egg number [-]	91	74
Egg weight [g]	58.96±4.91	61.50±5.20
Egg mass laid per chicken per day [g/d]	19.72	16.73
FCR (feed:egg)	6.16	7.28
Survivability [%]	100	100

Table 5.24: Nutritional parameters of the eggs in experiment 6 of the control and experimental group, according to analysis

	Control group	**Experimental group**
Vitamin A [mg/kg]	2.550	2.310
Vitamin E (Alpha-Tocopherol) [mg/kg]	40	40
Total fat [%]	11.6	9.5
Saturated FAs [%]	4.15	3.25
Monounsaturated FAs [%]	5.44	4.10
ω-6 FAs [%]	1.35	1.42
ω-3 FAs [%]	0.51	0.63
DHA [%]	0.05	0.06
ω-6/ω-3 ratio	2.65	2.25

5.6.3 Discussion and Conclusion

Higher laying productivity in T1 over T2 was observed, so that FCR was lower in T1, as both groups consumed the same amount of feed (table 5.23). Both groups lost body weight on average, the control group slightly more so.
The data in table 5.24 shows that the eggs from T2 having been fed twice the amount of flaxseed had the same amount of vitamin E and were lower in vitamin A and total fat content. They had more polyunsaturated FAs, both ω-6 and ω-3. The ω-6/ω-3 ratio in the T1 diet was 1.16 and 2.65 in the eggs. In T2 it was 0.78 in the diet and 2.25 in the eggs. In T1 the ω-6/ω-3 ratio was more than two times higher in the egg than in the diet, in T2 it was almost three times higher in the egg than in the diet. Apparently, the lower the ω-6/ω-3 ratio, the harder it gets to bring it further down by manipulating the diet. The DHA content in the eggs of T2 was higher than in T1, despite the lower total fat content.
Caston et al. (1994) observed decreased body weight and egg weight and significantly increased feed intake with increasing level of flax seed (10 and 20 %) in the diet of Single Comb White leghorn pullets compared to no flax seed in the control diet. Laying performance remained unaffected.
Cherian and Quezada (2016) fed Lohman Brown laying hens diets containing 0 and 10 % flax seed. Both egg production and feed intake increased in hens being fed flax seed. The ω-6/ω-3 ratio decreased from 5.99 to 2.80, while DHA content increased from 0.66 to 1.35 % of total lipids.
Cherian and Sim (1991) fed diets containing 0, 8 and 16 % flax seed to Single Comb White Leghorn laying hens. The diets contained a ω-6/ω-3 ratio of 7.05, 0.60 and 0.31, respectively. The corresponding eggs had ω-6/ω-3 ratios of 6.61, 1.80 and 1.29, respectively. The DHA content in the eggs was 1.02, 1.42 and 1.54 % of total fat content.
Hayat et al. (2009) noticed a slightly decreased feed intake, laying rate and FCR at 10 % flax seed in the diet of ISA Brown Leghorn laying hens relative to the control. Eggs had a ω-6/ω-3 ratio of 7.96 for the control and 3.42 flax seed group. The DHA percentage increased from 2.07 to 3.75 % of the total fat content in the eggs. They also found that both short and long-chain ω-3 FAs would be increased in the eggs, when the diet was supplemented with antioxidants (α-tocopherols and butylated hydroxy toluene).
The beneficial effect of flax seed in the diet of laying hens on the ω-6/ω-3 ratio is well documented, but the effects on laying performance are not consistent. Depression in the hen's body weights with increasing amounts of flax seed in the diet was a common

observation.
Caston et al. (1994) detected significantly higher levels of malondialdehyde in the liver of hens fed 20 % flax seed in their diet compared to 0 and 10 %. Malondialdehyde is a common marker for oxidative stress.
Duckweed can be used to decrease the ω-6/ω-3 ratio further, when the level of supplementation of flax seed is already at a maximum tolerable level. However, the effect of duckweed in changing the ω-6/ω-3 ratio is quite limited, when the diet is already supplemented with flax seed, as the effect gets much weaker especially when the ω-6/ω-3 ratio in the diet gets below 1:1.

5.7 Experiment 7

This trial investigated on the growth performance of grass carp being fed on duckweed only.

5.7.1 Materials and Methods

Twenty six grass carp (*Ctenopharyngodon idella*) adult fish (mean initial weight 77.17 g ± 19.48 g) were fed with a mixture of duckweed species including *Lemna gibba, Spirodela polyrrhiza* and *Wolffia arrhiza* and did not receive any supplemental feed. The fish were kept in an IBC tank that was connected to an aquaponics rig inside of a polytunnel, so the water was constantly being exchanged and aerated. Water temperature ranged from 22 to 26 °C during the trial. The fish were fed fresh duckweed *ad libitum* one or two times a day, so that there was always at least some duckweed floating in the fish tank. The fish were given 12 days to get accustomed to the conditions before the experiment started and only fed duckweed during this period and during the experiment. The fish were weighed at day 0, 9 and 17, all calculations are based on total fish body weight. The duckweed was weighed every time before it was dropped into the fish tank, after it was harvested and gravity-drained in a net.

5.7.2 Results

The growth performance of the grass carp are depicted in table 5.25

Table 5.25: Growth performance of grass carp being fed on duckweed only

	Day 0 - Day 9	Day 9 - Day 17	Day 0 - Day 17
Initial total fish weight [g]	1,930	2,280	1,930
Final total fish weight [g]	2,280	2,480	2,480
Total weight gain [g]	350	200	550
Duckweed fed FW [g]	19,300	14,700	34,000
Duckweed fed DM [g]	1,081	823	1,904
Duckweed consumed per day per fish weight [g/g/d] (FW basis)	1.02	0.77	0.91
Specific growth rate [%/d]	1.85	1.05	1.47
FCR	3.09	4.12	3.46

5.7.3 Discussion

The grass carp had a SGR of 1.47 %/d and a FCR of 3.46. Feed intake, SGR and FCR were all better during the first half of the trial. The average body weight was 77.17 g ± 19.48 g at day 0 and 99.20 ± 15.96 g at day 17.
Most research on duckweed fed fish has either been carried out in fertilized ponds, where duckweed was fed as a supplement and the fish mainly sustained on plankton [Kabir et al., 2009] [Skillicorn et al., 1993] [Chowdhury et al., 2008] or duckweed was included into pellets at different inclusion percentages [Tavares et al., 2008] [Fasakin et al., 2001] [Khandaker et al., 2007] [El-Shafai et al., 2004] [Fasakin et al., 1999].
The literature was also reviewed in chapter 4.1.3.
There is a small amount of research where fish have been fed duckweed exclusively [Tavares et al., 2008] [Pípalová, 2003] [Yibo et al., 1994] [Hassan and Edwards, 1992].
Hasan and Chakrabarti (2009) reviewed the literature on fish, mainly Nile tilapia and various carps that were fed with duckweed exclusively.
However, the big majority of this research has been carried out with fry or fingerlings. Fish are called fry when they are 1 -2 cm in length and fingerlings when they are 10 - 15 cm in length. The SGR, FCR and utilization capacity for the digestion of duckweed is likely to change as the fish mature. Consequently, data on growth characteristics of fingerlings can hardly be used to estimate the productivity of an aquaculture system, where fish are typically slaughtered at a body weight of 500 - 1,000 g.
Shireman et al. (1978) fed grass carp (*Ctenopharyngodon idella*) with duckweed (*Lemna minima*) only. They cultivated fingerlings (3 g) and adult fish (63 g) for 68 days. The fish had a SGR of 3.88 and 1.19 %/d and a FCR of 1.6 and 2.7, respectively. This shows that differences in fish body weight are affecting SGR and FCR.
Research on bigger fish fed duckweed is scarce.
Cassani et al. (1982) fed hybrid grass carp (*Ctenopharyngodon idella* X *Hypophthalmichthys nobilis*) of over 1 kg body weight with duckweed (*Lemna gibba* and *Wolffia columbiana*) for 60 days. The FCRs were 6.69 and 3.76 and SGRs were 0.21 and 0.51 %/d.

5.7.4 Conclusion

Duckweed can be the sole feed source for grass carp and still provide good growth characteristics not only for fingerlings. The integration of grass carp aquaculture into duckweed cultivation has the potential to be amongst the most efficient low-tech systems for the production of animal protein. Through the high biomass productivity of duckweed and the low FCR for duckweed-fed grass carp, this combination is highly suitable for resource-efficiency oriented aquaculture. Not only can this system be operated 100 % locally, but likely produces high quality fish with a well balanced ω-6/ω-6 ratio.

5.8 Experiment 8

In this trial the productivity of duckweed and *Azolla* and the feasibility of their cultivation was assessed.

5.8.1 Materials and Methods

Mixed species of duckweed were collected from the local drainage ditches containing *Spirodela polyrrhiza, Lemna gibba* and *Wolffia arrhiza. Azolla filiculoides* was obtained from a botanical garden. The duckweed (mixed species) and *Azolla* were grown in a

pond inside of a polytunnel and in a lined pond outside right next to the polytunnel, both receiving full sun. The water in both ponds was fertilized by the ducks that had access to the outer pond for 5 months before the floating plants were grown. Water from the outside pond was pumped into the pond in the polytunnel as a source of nutrients. Temperature and pH of the pond water were assessed once per week and total phosphorus, total nitrogen, ammonium-N and nitrate-N were measured every two weeks in both ponds.

Two floating rafts were constructed to enable the harvesting from a defined surface area in the ponds. Both rafts had 3 compartments to hold the duckweed, *Azolla* and a mixture of duckweed and *Azolla*, as illustrated in figure 5.4. The raft for the outside

Figure 5.4: Floating raft with separated compartments holding duckweed, duckweed/*Azolla* co-culture and *Azolla* (from left to right).

pond also had a chicken wire roofing to protect the floating plants from birds. The plants were placed in the compartments of the raft, so they would cover the whole compartment area of 0.25 m^2 on the 3rd April 2019. Harvesting began one day later and was continued every other day, when the plants were beginning to get visibly crowded. Floating plants were removed at a rate so that about 90 % of the compartment area remained covered. The harvested plants were drained in a sieve and weighed every harvest. Both duckweed and *Azolla* were sent into the lab for analysis (at the Institute of Food Quality of LUFA Nord-West), where DM content and crude protein content among other things were measured. The results were already shown in table 5.1.

5.8.2 Results

In figure 5.5 the course of the cumulative harvest over time can be seen. Table 5.26 shows the amount of floating plants that were harvested and the extrapolated yields of biomass and protein. In table 5.27 the temperature, pH, phosphate and nitrogen concentrations are given for the pond outside and in the polytunnel during the harvesting of the floating plants.

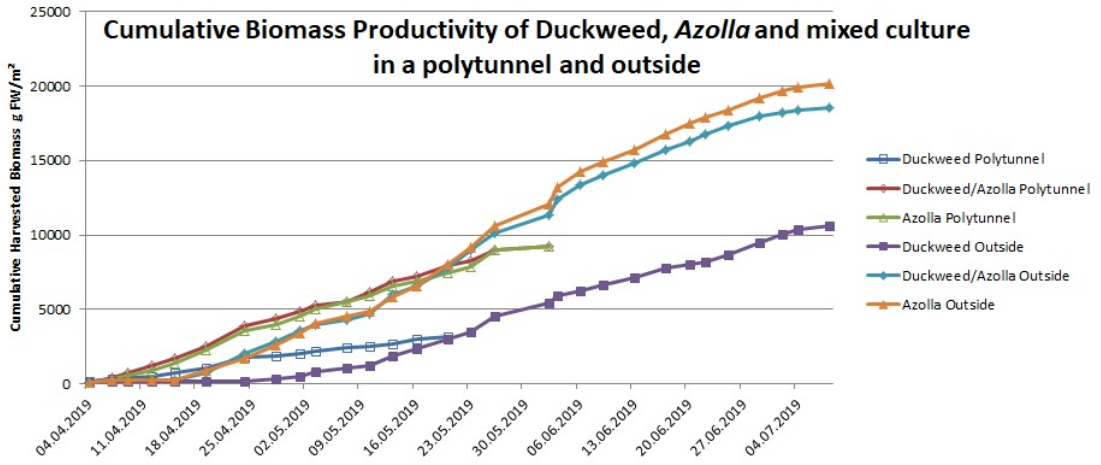

Figure 5.5: Cumulative yield of duckweed, duckweed/*Azolla* and *Azolla* in the pond in the polytunnel and outside.

Table 5.26: Harvest data and extrapolated biomass DM and crude protein yield of duckweed, duckweed/*Azolla* and *Azolla*. *The DM content and crude protein content of the mixed cultures were not analysed, but assumed to be identical to the *Azolla* monoculture.

	Polytunnel			Outside		
	Duck-weed	Duck-weed/ *Azolla*	*Azolla*	Duck-weed	Duck-weed/ *Azolla*	*Azolla*
Harvested Biomass [FW g]	798	2,311	2,312	2,649	4,628	5,038
Duration of Harvest [d]	46	59	59	95	95	95
Harvest Area [m²]	0.25	0.25	0.25	0.25	0.25	0.25
DM Content [%]	0.056	0.05*	0.05	0.056	0.05*	0.05
Extrapolated Productivity [t DM/ha/y]	14.2	28.6	28.6	22.8	35.6	38.7
Crude Protein Content [%]	0.337	0.293*	0.293	0.337	0.293*	0.293
Extrapolated Protein Productivity [crude protein t/ha/y]	4.78	8.38	8.38	7.68	10.42	11.34

Table 5.27: Pond water parameters during the harvesting of the floating plants from 04.04. to 08.07.2019. More details are shown in the Appendix D (table D.1).

	Temperature [°C]	pH [-]	Total Phosphate -P [mg/l]	Total Nitrogen -N [mg/l]	Ammonium -N [mg/l]	Nitrate -N [mg/l]
Outside	12.0-23.2	6.84-9.87	18.7-22.8	5.2-11.5	0.7-4.6	0.8-1.7
Polytunnel	15.8-32.8	6.94-7.35	16.5-20.7	2.4-10.2	0.6-2.5	1.0-2-7

5.8.3 Discussion

The *Azolla* monoculture was producing more than twice as much biomass than the duckweed did in the polytunnel pond and 1.7 times more in the outside pond. The duckweed/*Azolla* culture was inoculated as a mixture of 50 % duckweed and 50 % *Azolla*, but *Azolla* was soon making up the majority, as it grew faster than the duckweed and also

grew over the duckweed, thereby shading it. Therefore, the duckweed/*Azolla* co-culture produced almost the same amount of biomass harvest as the *Azolla* monoculture did. As can be seen in figure 5.5, in April all floating plants in the polytunnel grew faster than outside due to the temperature difference. During May, the productivity of the floating plants accelerated outside and overtook the ones in the polytunnel.
The phosphorus concentration was always higher than the nitrogen concentration in both ponds. This is due to the composition of the duck manure, but also due to nitrogen losses caused by microbial nitrification and denitrification and volatilization of ammonia, whereas phosphorus is usually not lost. The fact that the phosphorus concentration never exceeded 25 mg/l even though there was a constant input, suggests that insoluble phosphorus compounds were accumulating in the sediments of the pond, keeping the soluble phosphate concentration fairly stable. The total nitrogen concentration was rather low, what could have led to *Azolla* producing much more biomass than duckweed. While duckweed requires high reactive nitrogen concentrations for excessive growth, *Azolla* does not need any at all. Leng (1999) suggested that 20 mg ammonia-N/l is sufficient for optimal growth of duckweed.
However, Lasfar et al. (2007) found that *Lemna minor* was not limited in growth at a nitrogen concentration of 3 - 5 mg N/l.
Besides the concentration of nitrogen, the ratio of nitrogen to phosphorus or possibly other elements might also be a factor involved.
The outside pond had 11.5 mg total N/l and 4.6 mg ammonia-N/l as the highest readings and the polytunnel pond 10.2 mg total N/l and 2.5 mg ammonia-N.
The extrapolated yield of *Azolla* was 38.7 t DM/ha/y outside and 28.6 t DM/ha/y inside the polytunnel. For duckweed it was 22.8 t DM/ha/y outside and 14.2 t DM/ha/y. The polytunnel enabled the plants to start growing a few weeks earlier, but the plants were more susceptible to pests. In total, it did not provide any meaningful advantage over the outside pond. The yield in the outside pond was rather low for duckweed, however for*Azolla* it was quite high. The yield potentials of both plants were already discussed in chapter 4.1.1 and 4.2.1.
At the beginning of the harvest, growth was low due to cold temperature and at the end it was also lower due to pests. However, in between, just for the month of June, the extrapolated yield for *Azolla* and duckweed outside was 53.9 t DM/ha/y and 35.0 t DM/ha/y.
However, extrapolated yields can easily be overestimated due to longer day length in summer. Samples from both *Azolla* and duckweed were taken on the 3rd of June 2019 from the outside pond. The crude protein content was 29.3 % in *Azolla* and 33.7 % in duckweed. Due to higher productivity of *Azolla*, the protein productivity was substantially higher in *Azolla* than in duckweed.
The continuous harvest was stopped when the floating plants became so heavily infested with pests that they did not grow any more. Duckweed was first affected by caddisflies (Trichoptera). The larvae were feeding on the duckweed and then using the fronds to build their cocoon, as is shown in figure 5.6. The larvae completely cleared the whole compartment of duckweed in the polytunnel, the damage in the outside pond from caddisfly larvae was much less. Aphids were also present in very high numbers on both *Azolla* and duckweed outside and inside the polytunnel, as shown in figure 5.7. *Azolla* in the outside pond was also infested with what appeared to be the *Azolla* weevil (*Stenopelmus rufinasus*), which is also used as a biological pest control agent against unwanted *Azolla*. The infestation of multiple pests on the floating plants was so severe that most floating plants eventually died off. At one point, the outside pond only had *Wolffia arrhiza* growing in it that was apparently too small for the aphids to be attacked. The

Figure 5.6: Caddisfly larvae decimating duckweed and pupating inside a cocoon made of duckweed fronds.

Figure 5.7: Duckweed infested with Aphids. Note that they only sit on fronds that are bigger than *Wolffia arrhiza* eventually killing them off completely, while the latter species with the smallest fronds was not affected.

pest pressure was alleviated on its own after some weeks.
Interestingly, the very same mixture of duckweeds was growing locally in almost all drainage ditches, where pests were not visible, even just a few meters from the ponds away. *Azolla* was also grown in one ditch and did not seem affected by pests at all. The reasons for this phenomenon are unclear, but might have something to do with the liners used for the ponds. They might have acted as a barrier at the shore of the pond towards

pest predators. Lady bug larvae were observed in the outside pond, but apparently, they were not enough to control the aphid population.
An unnoticed infestation of duckweed or *Azolla* can induce a sudden and total collapse of the production. Frequent observations are therefore necessary. A vegetated natural shoreline of the ponds and adjacent trees might help the distribution of natural predators in the pond. Under this livestock production system, the low nitrogen concentration or the low N:P ratio likely favoured the integrated production of *Azolla* over duckweed in terms of productivity.
However, duckweed was shown to be a more beneficial supplementary feed for most animals judging from the feeding trials discussed in chapter 4.1.3 and 4.2.3, the practical experiments conducted (chapter 5) and also from the nutritional analysis done on *Azolla* and duckweed grown in the same pond in table 5.1. Duckweed had slightly less sugar than *Azolla*, but more crude protein, more starch and more fat, with higher amounts of polyunsaturated FAs at almost the same ω-6/ω-3 ratio.
Whether the cultivation of *Azolla* or duckweed is better suited for integrated feed production depends mainly on the composition of the nutrient input. If the total nitrogen content can be maintained at least 10 - 20 mg/l in the medium, duckweed cultivation will most probably be the better choice in most cases. *Azolla* is preferable, when there is a desire or need to restrict the use of synthetic nitrogen fertilizer or if available light is very limited.
Mixed culture of *Azolla* and duckweed is also possible, while *Azolla* might out-compete duckweed under low nitrogen concentrations and vice versa under high nitrogen concentrations in the medium.

5.8.4 Conclusion

Both floating plants duckweed and *Azolla* were shown to have very high yield potential, producing far more protein than conventional feed crops. Duckweed showed more promise in terms of feed quality, but *Azolla* could be better suited in high phosphorus, low nitrogen environments, which is very common for most types of manure.
Problems with pests were very severe in the lined ponds, but were not observed in the surrounding drainage ditches containing the same plants. Reasons for this observation are not clear, but might be caused by the liner covering the shore and preventing predatory insects from accessing the infested floating plants in the pond. Considering adequate pest control, both floating plants can produce a substantial amount of feed high in protein, lowering feeding costs and recycling liquid manure directly on-site.

5.9 Observations and Concluding Remarks

To put the space requirements for floating plant production into perspective, the following approximations are given using the results from the trials:
Fresh duckweed was consumed at 355 g per duck and 296 g per chicken per day. For *Azolla* it was 304 g and 73 g, respectively. With the extrapolated yield of 22.8 t DM/ha/y of duckweed established over 95 days, 3.2 m^2 of pond area would be needed per duck and 2.7 m^2 per chicken. For *Azolla* that showed a yield of 38.7 t DM/ha/y it would be 1.4 m^2 per duck and 0.3 m^2 per chicken.
This would allow for the production of floating plants at a rate that the animals were observed to consume in the trials. Of course this is only possible during the growing season or when the water temperature is approximately over 15°C.
The feeding trials had several weaknesses. The floating plants were only analysed once

and these values were used for all calculations. It is very likely that the nutritional profile of the plants was changing throughout the trials. The amount and type of feed that the ducks and chickens were acquiring from the pasture is unknown.
The ω-6/ω-3 ratio in the eggs of the six trials can be seen in figure 5.8. The most

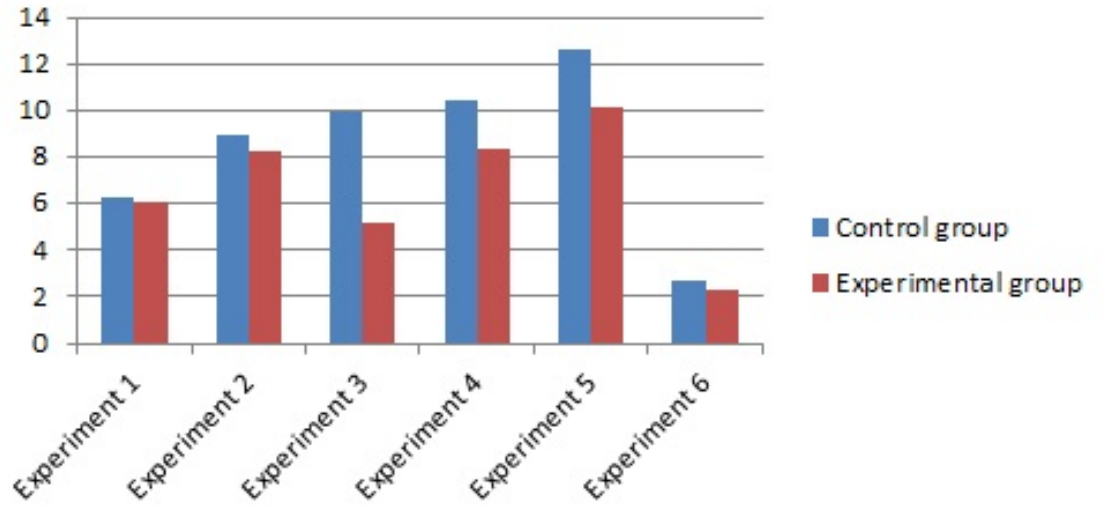

Figure 5.8: The ω-6/ω-3 ratio in the duck and chicken eggs from the six trials.

significant difference between control group and experimental group was observed in Experiment 3, when ducks were fed duckweed. *Azolla* in the diet of the ducks was less effective at changing the ω-6/ω-3 ratio. However, for the chickens apparently the offering of *Azolla* had more impact on the ω-6/ω-3 ratio than duckweed did, even though duckweed was consumed in substantially higher amounts.
The offering of floating plants to ducks without changing the concentrate feed showed the least effect on the ω-6/ω-3 ratio. In Experiment 6, the difference in the ω-6/ω-3 ratio between the two groups (5 % vs. 10 % flaxseed) was relatively small. The effect of flaxseed in the diet on the ω-6/ω-3 ratio was however much more pronounced than any floating plant was, when comparing the trials.
Both *Azolla* and duckweed showed less potential in altering the ω-6/ω-3 ratio than flaxseed, however they can decrease the feeding costs by providing protein and pigments, decrease dependence on imported feed stuff and increase overall efficiency and sustainability by closing local nutrient cycles.

Table 5.28 shows the feed consumption per produced egg mass in Experiment 2 to 5. The consumption of wheat was increased when ducks and chickens were fed with *Azolla*, but the consumption of supplementary concentrate, was always reduced by at least 20 % in the experimental groups. The offering of duckweed reduced the consumption of both wheat and supplementary concentrate. On average, the consumption of wheat was reduced by 5.2 % and supplementary concentrate by 33.6 % per egg mass produced when floating plants were offered. Floating plants are primarily a substitute for the feed components high in protein, such as soybean meal or oil seed cakes (including added micronutrients) that are substantially more expensive than the grain components such as wheat or corn.

The ducks consumed more duckweed and *Azolla* than the chickens did, both in terms of absolute amount and in relation to the concentrate feed. The ducks' eggs also tended to have a lower ω-6/ω-3 ratio and a higher ω-3 FA percentage than chicken eggs, even

Table 5.28: Feed consumption of the birds per produced egg mass [g DM/g FW] in Experiment 2 and 3 (with ducks) and Experiment 4 and 5 (with chickens).

	Control Group [g/g]	**Experimental Group** [g/g]	**Relative Difference** [%]
Experiment 2			
Wheat	3.17	3.62	+14.2
Suppl. Conc.	3.41	2.72	-20.2
Azolla	-	0.82	-
Experiment 3			
Wheat	3.91	3.46	-11.5
Suppl. Conc.	4.19	2.60	-37.9
Duckweed	-	1.32	-
Experiment 4			
Wheat	1.95	2.15	+10.3
Suppl. Conc.	2.10	1.62	-22.9
Duckweed	-	0.78	-
Experiment 5			
Wheat	5.01	3.32	-33.7
Suppl. Conc.	5.37	2.50	-53.4
Azolla	-	0.20	-

though this might have been influenced by the season, as the chickens were exposed to cooler temperatures than the ducks. Judging from the potential improvement of the egg's quality through feeding of floating plants, ducks showed more promise than chickens.

The combination of ducks and ponds however could lead to several problems in larger scale production. In the first feeding trial, many ducks continuously laid their eggs into the pond, where they simply sank to the ground. They also spent most of their time in the pond, so that the nutrient load from duck manure was much higher than the nutrients removed through the feeding of floating plants.

The grass carp showed more promise in the utilization of floating plants than ducks and chickens. Cai and Curtis (1989) showed that grass carp that were fed exclusively with aquatic plants, containing no DHA, still had a DHA content of over 8 % in their fat tissue, more than 10-fold higher compared to what was measured in duck's or chickens' eggs. Because of that and the fact that grass carp can be fed 100 % with duckweed, they are clearly superior in turning floating plants into a high quality animal product than ducks or chickens.

The grass carp were also fed with other floating plants after the trial ended and their preference was observed to be in the following order (starting with the least favoured plant): water lettuce (*Pistia stratiotes*) < Amazon frogbit (*Limnobium laevigatum*) < *Azolla filiculoides* < Duckweed (*Spirodela polyrrhiza, Lemna gibba* and *Wolffia arrhiza*).

Free-range chickens on the farm that were not confined during the day, were eagerly consuming fresh *Azolla* when offered, even though they had access to concentrate feed and herbs and grasses. They were also observed to pick duckweed directly out of a drainage ditch on their own. Both free-range chickens and ducks can be provided with fresh floating plants just by constructing a pond of sufficient size. The water quality of the pond has to be monitored with ducks, as they will spend most of their time in the pond supplying a high nutrient load.

A pond with floating plants at the right size will produce more usable protein for poultry than any pasture or feed crop per surface area. While the pasture will be damaged by the chickens depending on the flocking density and grazing duration, a pond can not be

damaged in such a way and provides feed without the need to rotate the chickens on different areas.
Floating-plant production systems have the capacity to improve nutrient efficiency compared to conventional feed production that is associated with environmental pollution from agricultural run-off.

6. Case Study

6.1 Goal Setting

This case study will be used to illustrate the feasibility of a floating plant-based aquaculture for tropical/sub-tropical climates. The goal is to create a highly productive system capable of producing animal protein with minimized land consumption and environmental impact with local resources only.
The duckweed-aquaculture presented by Skillicorn et al. (1993) will be used as a role model, as their system was already, proven by several years of operation, at the front of sustainable aquaculture in terms of protein productivity and resource efficiency, as already discussed in chapter 4.1.4.
The combination of duckweed and fish is likely to be one of the most efficient ways of producing animal protein, as duckweed is among the fastest growing plants and fish, as cold-blooded animals have a low FCR compared to other livestock and some have the ability to handle a 100 % duckweed diet. However, there is still room for improvement on several aspects concerning the duckweed aquaculture system after Skillicorn et al. (1993):

Adequate Nutrient Supply
Skillicorn et al. (1993) reported a duckweed yield (*Spirodela, Lemna* and *Wolffia* species) of 13 - 38 t DM/ha/y. Mohedano et al. (2012) reported a yield of 68.8 t DM/ha/y of duckweed (*Landoltia punctata*) under realistic conditions for one year (see chapter 4.1.4). The reason for this discrepancy is likely to be found in an inadequate fertilizing regime. Skillicorn et al. (1993) used NPK fertilizer combined with sea salt as a source of trace elements. The authors stated: "The most important immediate research priority to advance duckweed production is to determine fertilizer requirements, particularly nitrogen and trace elements. The current practice of using of urea and unrefined sea salt is clearly inadequate."
Mohedano et al. (2012) was using digester effluent of pig waste that was apparently a more complete nutrient source supporting continued high growth rates of the duckweed. Therefore the suggested system will rely on frequent analysis of water, duckweed and fish to ensure adequate nutrient supply of all required elements for high growth rates.

Oxygen Supply
Skillicorn et al. (1993) used non-aerated fish ponds, which were able to hold a fish stocking density of 10 - 15 t/ha at most. Higher stocking densities were not possible due to limited amounts of dissolved oxygen. This is not only caused by the fish themselves, but also by bacteria decomposing the fish manure at the pond bottom and phytoplankton suspended in the water column, such as micro algae prolifically growing in the pond due to high nutrient conditions. While the phytoplankton produces oxygen during the day through photosynthesis, it will deplete oxygen during the night.

For the suggested system, by keeping the fish in cages in the same pond as the duckweed, phytoplankton can hardly grow, as the duckweed is blocking the light at the water surface. The duckweed will not compete for oxygen with the fish at night, as the stomata of duckweed are located on the upper side of the fronds [Leng, 1999].

Nutrient Recycling
In the system described by Skillcorn et al. (1993), the fish manure stays in the fish pond, where it is decomposed by bacteria. Phytoplankton is taking up the nutrients being released and serves as a nutrient source for the fish, additionally to the duckweed. However, high concentrations of phytoplankton are elevating the pH of the pond water during the day, which can result in an increase from 7 up to over 9 [Shammas et al., 2009]. This is shifting the equilibrium of ammonium to ammonia towards the latter, which is toxic to the fish and substantially increases nitrogen losses from the system through volatilization of ammonia.
This pH shift does not occur in the duckweed pond, as the duckweed inhibits algal growth by blocking the light, which also decreases water temperature in hot climates. Hence, the production of duckweed is much less nitrogen wasting as the production of phytoplankton in open ponds. The less phytoplankton is growing in the system, the less nitrogen is lost.
The system described by Skillicorn et al. (1993) uses 0.5 ha of duckweed pond area to feed fish in a 1 ha fish pond, with high density of phytoplankton as the fish manure does not get removed.
In the suggested system, by keeping the fish inside the duckweed pond, nitrogen efficiency will be greatly improved. Additionally, all other elements in the fish manure can directly be recycled by the duckweed.
Water quality will be improved by combining the fish pond and the duckweed pond, as it enables direct nutrient recycling and provides more total water volume for the fish, so that water quality parameters are more stable.

Reducing Manual Labour
About one ton of fresh duckweed per hectare was harvested daily by hand and carried to the fish pond as described by Skillicorn et al. (1993). This can easily be circumvented by having the fish in the duckweed pond.
Manual labour (or pump work) is still required in the suggested system to push the duckweed mats on the water surface above the fish cages, but there is no manual lifting or carrying of the duckweed involved.
Using pumps, the feeding might be automated completely by circulating the duckweed mats in raceway ponds.

6.2 Basic Set-up of the Suggested System

The suggested improved system consists of a duckweed pond with integrated fish cages, as shown in figure 6.1. The duckweed mats in the raceway ponds will be moved into the fish cages, that are constructed out of mesh wire. The mesh size is 2.0 cm, so that duckweed can pass through, but the fish are retained. Fertilizer is applied frequently to avoid damaging the fish. The solar pump will only run during the day to facilitate better nutrient distribution.
With a strong enough pump, automated feeding might be possible, but prior field testing is required.
Mohedano et al. (2012) obtained a duckweed yield of 68.8 t DM/ha/y. In this set-up,

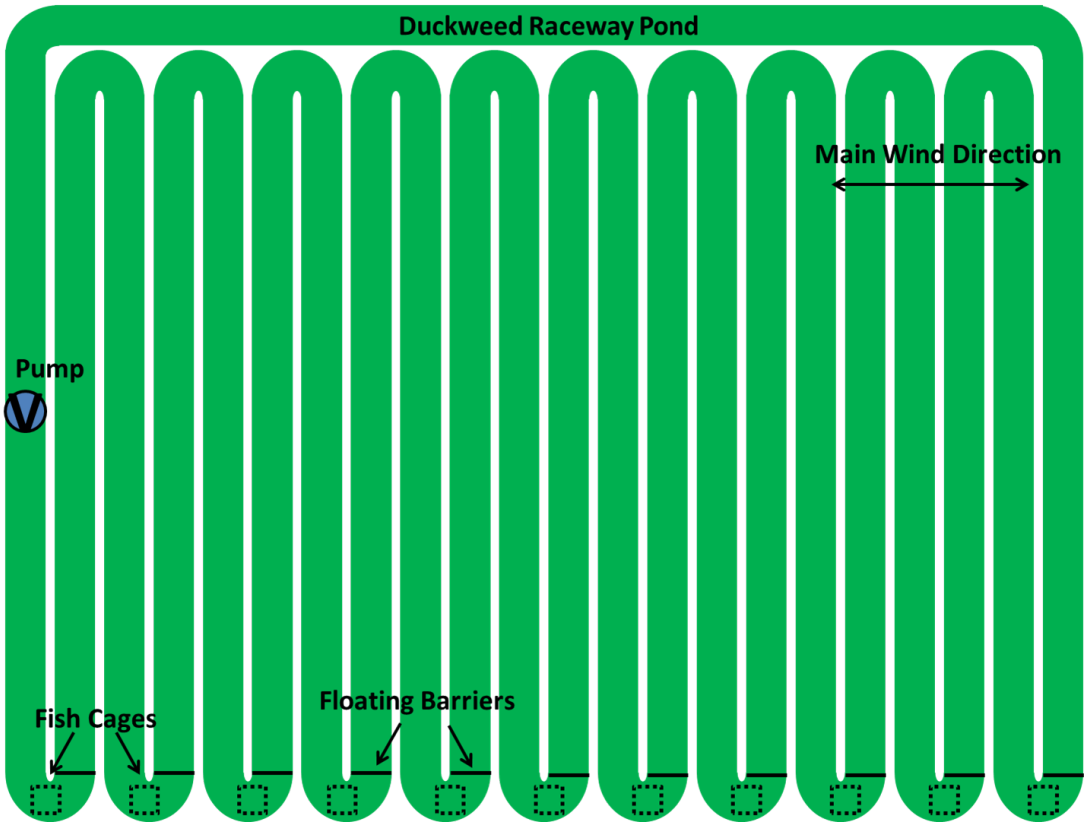

Figure 6.1: Layout of the duckweed raceway pond, 0.5 m deep, covering 4.368 ha of which 3.468 ha is pond area. The pond channels are 9 m wide and the space between the channels is 2 m wide. The fish cages are boxes out of mesh wire, 6 m long, 6 m wide and 0.5 m high, in the lower turning points, for easier harvest. One solar pump is circulating the water to distribute nutrients and move the floating duckweed mat. Floating barriers are retaining the duckweed mats going with the stream. The areas between the channels is planted with crops to provide wind breaks.

the yield is assumed at 50 t DM/ha/y. In order to calculate the water volume needed for the fish, the FCR, SGR and stocking density is needed.
Nile tilapia are used for this system, due to their popularity and ability to feed on duckweed alone. The FCR increases with increasing fish bodyweight. The slaughter weight of Nile tilapia is assumed to be 500 g. The FCR can be as low as 1.0 for small duckweed-fed fingerlings [Hasan and Chakrabarti, 2009], but values for market size Nile tilapia could not be found. However, a FCR of 3.76 and 6.69 was observed by Cassani et al. (1982) with duckweed-fed grass carp of over 1 kg bodyweight, depending on duckweed species fed.
An average FCR of 5 will be assumed for the Nile tilapia of mixed sizes, from fingerlings to 500 g of body weight.
There is also hardly any information on the SGR of duckweed-fed Nile tilapia. Fasakin et al. (2001) found a SGR of 0.8 % for duckweed-fed Nile tilapia fingerlings. Hassan and Edwards (1992) found a SGR of 1.34 %/d in duckweed fed Nile tilapia at an initial weight of 43.7 g and a final weight of 111.4 g during a period of 70 days. Cassani et al. (1982) found a SGR of 0.51 and 0.21 % with duckweed-fed grass carp with over 1 kg body weight. An average SGR for the Nile tilapia in the proposed system will be assumed at 1.0 %.

The stocking density is mainly depending on water quality, especially dissolved oxygen levels. In this system, water inside the cage will be replaced continuously, allowing for a higher stocking density. With Nile tilapia under intensive conditions, stocking densities can be 140 kg/m³ (total fish mass FW per volume of water) or higher [Hargreaves et al., 1991]. In the proposed system, the stocking density will be around 48 kg/m³.
With the proposed parameters, the standing biomass of fish is at 2.74 t, taking up 114.2 m² of 1 ha of total pond area and producing a harvest yield of 10.00 t FW fish per year, as shown in table 6.1.

Table 6.1: Parameters of the basic duckweed-based aquaculture set-up

	Fish Cages	**Duckweed Pond**
Organism	Nile tilapia	Duckweed species
Area [m²]	114.20	10,000.00
Depth [m]	0.50	0.50
Volume [m³]	57.10	5,000.00
Mat Density FW [kg/m²]	-	1.00
Stocking Density FW [kg/m³]	47.98	-
DM Content [%]	22.00	5.00
Standing Biomass DM [t]	0.60	0.50
Standing Biomass FW [t]	2.74	10.00
Feeding Rate [FW t/FW t/d]	1.00	-
SGR [%]	1.00	27.40
FCR [-]	5.00	-
Annual DM Output [t]	2.20	50.00
Annual FW Output [t]	10.00	1,000.00

The area required for the fish pond in proportion to the duckweed pond area in the suggested system is much smaller compared to the original system described by Skillicorn et al. (1993) as shown in figure 6.2. The duckweed pond is arranged in a serpentine as opposed to a square surface in order to facilitate easier harvest.
It also prevents the duckweed from being blown against the shore, where it can be piled up and rot. As shown in figure 6.3, banana plantations and other crops on the embankments between the serpentines can reduce the wind speed on the pond surface and stabilize the embankments.

If there is no access to fresh water supply, rain water harvesting methods can be employed. Deep storage ponds can be constructed at the lowest point in the area to collect run-off from heavy rains and store the water to pump it into the duckweed pond when needed for a steady water supply. The storage pond can be used to cultivate *Azolla* to reduce evaporation, control the spreading of mosquitoes and fix nitrogen [Hügel, 2017].

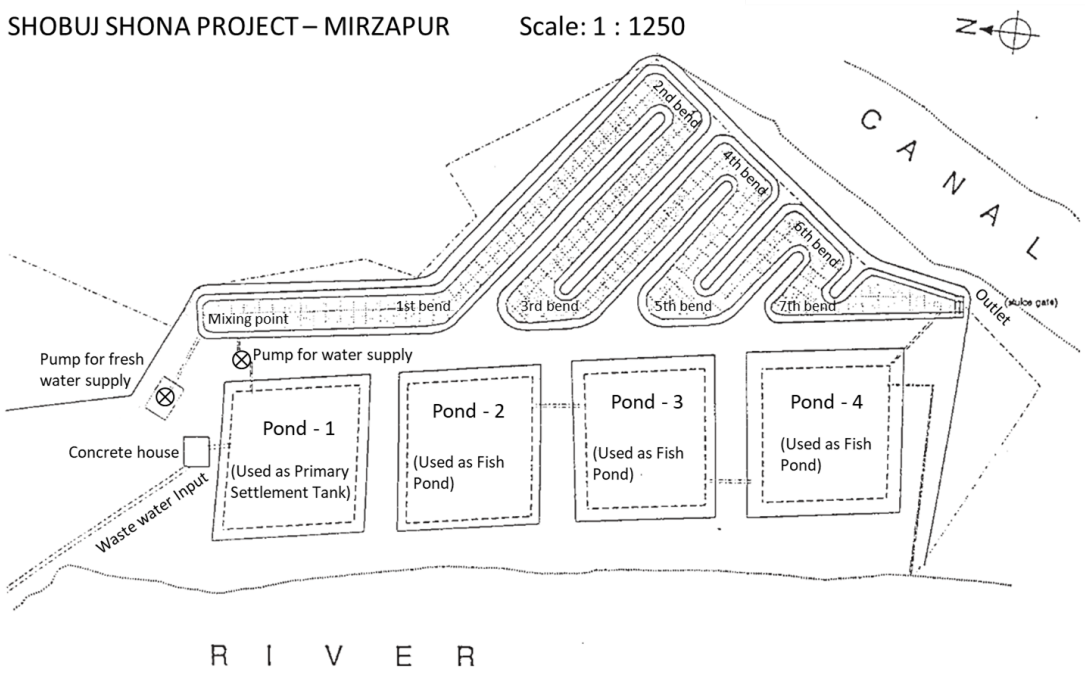

Figure 6.2: Layout of duckweed-covered serpentine plug-flow lagoon, anaerobic sedimentation pond (Pond-1), and fish ponds (Pond-2, Pond-3, Pond-4) at Mirzapur demo farm of PRISM Bangladesh. Each pond has an area of 0.2 ha, from [Iqbal, 1999](modified inscriptions).

Figure 6.3: Groundwater/chemical fertiliser-based duckweed cultivation of PRISM Bangladesh at Mirzapur demo farm complex. Duckweed ponds with floating bamboo grids and co-crops on the embankments in the foreground, and main fish production pond in the background, from [Iqbal, 1999].

6.3 Water and Nutrient Balance

A daily water loss of 6 mm on the water surface through evapotranspiration and seepage is assumed. With an annual rainfall of 1,095 mm, the average daily net loss is at 3 mm, which corresponds to a yearly water loss of 10,950 m^3/ha in the pond. This water needs to be replaced with fresh water, so 39.14 m^3 need to be fed into the pond daily at any point, preferably pointing into the same direction as the pump.

The amount of nutrients that need to be added to the system is at least as much as the nutrients contained in the harvested fish taken out of the system, as shown in table 6.2.

The majority of the different elements will be recycled inside of the system in the form

Table 6.2: Elemental composition of Nile tilapia after [Gonzales and Brown, 2006], layer chicken manure, feeder pig manure and dairy cow manure after [Jensen, 2013] (for N) and [Sager, 2007] (for S) and after [Sheppard and Sanipelli, 2012] (for the remaining elements) and human urine after [Bouatra et al., 2013]. The nutrient sources are also given as percentage of the Nile tilapia in "(%)" for each element to illustrate their potential as a fertilizer to replenish the nutrients lost from the system through the fish harvest. All elements essential for plants and animals are included.
[a]values are estimated
[b]N calculated from urea, creatinine and ammonia

Element	**Nile Tilapia [mg/kg DM]**	**Layer Chicken Manure [mg/kg DM]**	**Feeder Pig Manure [mg/kg DM]**	**Dairy Cow Manure [mg/kg DM]**	**Human Urine [mg/l FW]**
N	93,000	64,000 (69 %)	72,727 (78 %)	52,941 (57 %)	15,342[b] (16 %)
S	6,000	3,500 (58 %)	6,000 (100 %)	5,100 (85 %)	n.a.
Ca	4,761.5	103,000 (2163 %)	25,100 (527 %)	26,500 (557 %)	235 (5 %)
Na	394.75	4,300 (1,089 %)	1,240 (314 %)	2,100 (532 %)	14,679 (3,718 %)
Cl	394.75[a]	65,900 (16,694 %)	1,970 (499 %)	3,800 (963 %)	10,353 (2,623 %)
P	258.7	16,800 (6,494 %)	17,200 (6,649 %)	7,600 (2,938 %)	2,116 (818 %)
Mg	127.5	6,600 (5,176 %)	1,400 (1,098 %)	9,900 (7,765 %)	308 (242 %)
K	56.85	26,900 (47,318 %)	34,200 (60,158 %)	7,700 (13,544 %)	4,227 (7,435 %)
Zn	13.45	518 (3,851 %)	103 (766 %)	350 (2,602 %)	0.54 (4 %)
I	7.05[a]	3.31 (47 %)	0.708 (10 %)	1.66 (24 %)	n.a.
Se	7.05	2.94 (42 %)	2.6 (37 %)	1.16 (16 %)	0.18 (2 %)
Mo	5.72	7.69 (134 %)	6.9 (121 %)	4.55 (80 %)	0.06 (1 %)
Co	0.62	1.52 (245 %)	1.23 (198 %)	1.61 (260 %)	n.a.
Cu	0.53	75 (14,151 %)	201 (37,925 %)	75.7 (14,283 %)	0.02 (4 %)
Ni	0.53[a]	6.91 (1,304 %)	5.73 (1,081 %)	4.59 (866 %)	0.01 (2 %)
B	0.38	36.3 (9,553 %)	33.4 (8,789 %)	24.3 (6,395 %)	n.a.
Fe	0.33	1,134 (343,636 %)	203 (61,515 %)	879 (266,364 %)	0.10 (32 %)
Mn	0.26	630 (242,308 %)	493 (189,615 %)	311 (119,615 %)	0.02 (6 %)
Cr	0.07	5.8 (8,286 %)	4.64 (6,629 %)	3.21 (4,586 %)	0.01 (14 %)

of fish, fish manure, bacteria, detritus, floating plants and as dissolved ions in the water, while only a fraction will be taken out in form of the fish harvest.
In table 6.3, a fertilizing regime is depicted to replenish all or most of the important elements for the harvest of 10 t (FW) of fish according to their composition found by Gonzales and Brown (2008), also depicted in table 6.2.

All essential elements for both plants and animals should be analysed in the fish tissue and duckweed samples frequently according to table 6.2 and also 4.1 and 4.2, as a deficiency of at least any of those elements might diminish the overall productivity of

Table 6.3: Example of a calculated complete chemical fertilizing regime with market prices for the production of 10 t of Nile tilapia (FW). With a total of 687 kg of N, which is multiple times more than the nitrogen loss from harvest (205 kg) to compensate for volatilization losses. Costs for trace elements are estimated.

Chemical Fertilizer	**Amount [kg]**	**Price per ton [$/t]**	**Costs [$]**
UAN Liquid Fertilizer (32 % N)	2,000	200	400
Potassium Sulphate (45 % K, 18 % S)	100	500	50
Ammonium Sulphate (24 % S, 21 % N)	100	150	15
DAP (23 % P, 21 % N)	100	450	45
Calcium Ammonium Nitrate (34 % Ca, 10 % N)	50	300	15
Magnesium Sulphate (13 % S, 10 % Mg)	50	120	6
Sea Salt (55 % Cl, 31 % Na)	50	160	8
Trace Elements (Fe, Mn, Zn, B, Cu, I, Se, Cr, Ni, Mo, Co)	25	2,000	50
Total	2,475		589

the system or the nutritional quality of the fish.
Selenium, cobalt, iodine, chromium and sodium are essential for animals, but not for plants. Still, provided the objective is to produce high quality food, elements regarded essential for humans have to be present in food products in sufficient amounts, regardless of their essentiality to plants.
According to an FAO source, fluorine, tin, vanadium and silicon are also considered essential trace elements for fish and shrimps [Tacon, 1987].
Takeuchi et la. (2002) fed two different diets to Nile tilapia, analysing some elements in the diets and the corresponding fish as shown in table 6.4. Apparently, the differences in elemental concentrations in the diets are much more pronounced than the differences in the fish. Hence, the fish were able to regulate their excretion of these elements according to their intake.
For instance, the control group received 32.7 mg/kg of calcium in their diet and the experimental group just 2.3 mg/kg. However, both groups of fish had very similar amounts of calcium in their bodies. The fish from the experimental group had lower growth rates compared to the control group, which might indicate a calcium and possibly also a phosphorus deficiency in the diet.
Collecting data sets of both fish and their feed in a dynamic system frequently could allow for assessing the preferred ratios of all dietary elements considered essential or beneficial using basic mathematical models. In the ideal case, the fish, the duckweed and pond water samples are analysed simultaneously to get a deeper understanding of the interrelation of those three system components and enable precise nutrient balancing for improved product quality and system longevity and productivity.
For instance, if a lack of selenium is detected in the fish, it can be introduced in water soluble form into the system to be taken up by the duckweed and then passed on to the fish through the food chain until the deficiency is no longer present in the fish.
This would not be an exact method, but rather a way of correcting an imbalance starting with the element lacking the most.

Instead of chemical fertilizer, organic fertilizers can be used as well. Table 6.2 shows the elemental composition of Nile tilapia, different types of manure and human urine on a DM basis. To replenish elements lost through the fish harvest, manures can supply

Table 6.4: Elemental composition of a commercial (control) diet and a spirulina (experimental) diet for Nile tilapia and the corresponding whole body composition of the fish resulting from that particular diet, on a DM basis, after [Takeuchi et al., 2002]. Calculated differences in elemental concentrations for both diets and fish from control and experimental group are included.

	Control Diet [mg/kg]	**Control Fish [mg/kg]**	**Experimental Diet [mg/kg]**	**Experimental Fish [mg/kg]**	**Diet Difference [mg/kg]**	**Fish Difference [mg/kg]**
P	23.6	24.4	13.4	29.2	10.2	-4.8
Ca	32.7	43	2.3	39.8	30.4	3.2
Mg	2.8	1.6	4.1	1.6	-1.3	0
K	10.6	14.1	13.5	14.2	-2.9	-0.1
Na	9	4.5	2.4	4.3	6.6	0.2
Cu	0.0129	0.0021	0.0028	0.0019	0.0101	0.0002
Fe	0.349	0.0906	0.4073	0.1125	-0.0583	-0.0219
Mn	0.0714	0.0032	0.0282	0.0028	0.0432	0.0004
Zn	0.1492	0.1155	0.0338	0.0956	0.1154	0.0199

most trace elements manifold, also major minerals, especially phosphorus and potassium. However, sulphur and nitrogen are under-represented in manures and urine as well. Urine is also a poor source of trace elements.
If organic fertilizers are used, they either have to be combined with nitrogen and sulphur fertilizers or they must be supplied in very high doses, which could lead to nutrient imbalances.
Sulphur can be supplied as elemental sulphur powder, as a slow-release fertilizer, while several alternatives exist for nitrogen supply covered in chapter 6.5.
However, these values can only serve as an orientation. The minimum requirements of all nutrients to rule out any nutrient-limitation is not known.
As a suggestion for a management tool, a practical way to determine some of these values, would be to analyse duckweed samples that are taken from cultures that are confirmed to have very high growth rates, ruling out any severe nutrient limitations. The idea is to find out what element is lacking the most, so that it can be added individually.
Additionally, nitrogen losses through ammonia volatilization and nitrification and denitrification processes need to be taken into account. These losses mainly depend on water pH, temperature, dissolved oxygen and ammonia/ammonium and nitrate levels.
Mohedano et al. (2012) reported that nitrogen losses through nitrification and denitrification processes were 72 % in one duckweed pond and only 4 % in another pond with lower nutrient concentrations.
The total nitrogen concentration in the duckweed pond should be kept at around 30 mg N/l, to ensure high growth rates and high protein content of the duckweed. Therefore, at the beginning of setting up the duckweed culture, at least 150 kg of nitrogen need to be added per hectare of duckweed pond area at a water depth of 50 cm, assuming reactive nitrogen-free water. Higher total nitrogen concentrations might not increase growth, but lead to higher nitrogen losses from the system or could harm the fish due to ammonia toxicity.
Besides nitrogen, all other elements need to be monitored. When no pond liner is used, but earthen ponds, certain elements coming from the soil can be taken up by the duckweed. Others might form insoluble precipitates and stay in the sediments of the pond bottom, like iron phosphate for instance. Therefore, a general fertilization protocol can

not be provided, as individual needs are arising out of the particular environmental conditions.
Fertilizing regimes need to be developed for any particular system over the course of operation. The longer a system is running, more data is available and the more precisely it can be fertilized.
The fertilizer, especially the nitrogen containing ones should be supplied as frequently as possible in divided doses to minimize the potential risk of damaging the fish by ammonia toxicity or oxygen depletion through nitrification.

6.4 Cost Balance

Table 6.5 shows the cost balance for the described duckweed-based tilapia production. Tilapia fillet yield is assumed at 33 % of total fish FW.

Table 6.5: Cost balance based on a pond area of 1 ha, producing 10 t (FW) of Nile tilapia per year. Numbers based on estimates for Paraguay, without taxes.

	Amount	**Price per Unit**	**Total Cost**
Capital Costs			
Land (for 1 ha pond area)	1.26 ha	3,000 $/ha	3,780 $
Earthworks	1,500 m^3	2 $/$m^3$	3,000 $
Solar Panel (12 V, 130 W, 0.8 m^2)	20 (16 m^2)	75 $	1,500 $
Voltage Converter (2,500 W, 12 V/230 V)		200 $	360 $
Water Pump (230 V, 750 W, 19,000 m^3/l)	3	150 $	450 $
Other Equipment			2,000 $
Duckweed Seed	1 t		200 $
Total			11,290 $
Recurrent Costs			
Fingerlings	22,000	0.1 $	2,200 $
Labour	1,000	3.5 $/h	3,500 $
Lab Testing			500 $
Fertilizer	3.5 t		789 $
Selling Expenses Tilapia Fillet	3,300 kg	4 $/kg	13,200 $
Total			20,189 $
Income Sales Tilapia Fillet	3,300 kg	8 $/kg	26,400 $
Net Income Per Year			6,211 $
Break Even Point	1.8 years		

6.5 Suggestion of System Adoptions for Alternative Nitrogen Sources

The use of synthetic nitrogen fertilizer can be problematic, due to high volatility seen with high concentrations. Regulations in organic agriculture, environmental safety con-

cerns, energy consumption, availability and costs might also be reasons to look out for alternatives. Several options exist to reduce or circumvent the use of synthetic nitrogen:

Azolla

The needed reactive nitrogen can be fully supplied by *Azolla*, fixing up to 1,200 kg N/ha/y [Brouwer et al., 2017]. The downside to this approach is that *Azolla* is clearly inferior to duckweed as fish feed, as discussed in the chapters 4.1.3 and 4.2.3. Therefore, *Azolla* can only be part of the Nile tilapia´s diet, the percentage here is assumed at 12.3 % at DM basis, the rest is duckweed. The area needed for *Azolla* will be at 16 % of the original duckweed pond area, as shown in table 6.6.

All the reactive nitrogen will come from *Azolla* that is fed to the fish, that will then

Table 6.6: Parameters of the duckweed and *Azolla* based aquaculture set-up

	Fish Cages	**Duckweed Pond**	***Azolla* Pond**
Organism	Nile tilapia	Duckweed	*Azolla*
Area [m²]	144.20	8,400.00	1,600.00
Depth [m]	0.50	0.50	0.50
Volume [m³]	57.10	4,200.00	800.00
Mat Density FW [kg/m²]	-	1.00	1.00
Stocking Density FW [kg/m³]	43.76	-	-
DM Content [%]	22.00	5.00	5.00
Standing Biomass DM [t]	0.55	0.42	0.08
Standing Biomass FW [t]	2.50	8.40	1.60
Feeding Rate [FW t/FW t/d]	1.00		
SGR [%]	0.83	26.10	19.21
FCR [-]	6.00	-	-
Annual DM Output [t]	1.67	40.00	5.60
Annual FW Output [t]	7.60	800.00	112.00
Nitrogen Budget [kg N/y]	-155.50	-	192.00
Nitrogen Fixation Rate [kg N/ha/y]	-	-	1,200.00

excrete the nitrogen, which is then used to fertilize the duckweed. The duckweed pond and the *Azolla* should be separated from each other, in order to minimize reactive nitrogen entering the *Azolla* pond. This would be wasteful, as it would decrease nitrogen fixation activity in the *Azolla* and unnecessarily compete with duckweed for available reactive nitrogen.

There is no research on the effect of feeding both duckweed and *Azolla*, but as shown in table 6.6, it is assumed that FCR will be higher and SGR lower.

Also, the duckweed productivity will be lower, due to less reactive nitrogen in the system. All factors combined, this will lead to a lower fish yield per area, but allows for a nitrogen fertilizer independent system. All other nutrients lost by harvesting the fish, besides nitrogen still need to be added.

Fish Slaughter Waste Recycling

Another option is to process the fish right after the slaughtering and feed the fresh slaughter waste such as fish heads, guts, fins, bones etc. back to the tilapia in the cages. While intra-species recycling is a serious health hazard for most livestock, fish in general are not susceptible to this practice [Cheng and Lo, 2016].

The fillet (skinless) percentage of the whole fish lies at 33 %, dressing percentage without head at 66 % and with the head at 86 % [Racocy, 2009].
Depending on how the fish are being processed and sold, up to 67 % of the total fish harvest (FW) can be fed back to the fish.
FCR and SGR of the Nile tilapia would likely be improved by incorporating animal protein into their diet. At the same time, the need for fertilizer will be reduced greatly. The productivity in terms of fish output could be improved by an estimated 4 %, as shown in table 6.7.
The fresh slaughter waste has to be ground to be fed to the fish, ideally in a processing unit directly above the fish cage.

Table 6.7: Parameters of the duckweed based aquaculture set-up with fish slaughter waste recycling

	Fish Cages	**Duckweed Pond**	**Slaughter Waste Recycling**
Organism	Nile tilapia	Duckweed	Nile Tilapia
Area [m²]	114.20	10,000.00	-
Depth [m]	0.5	0.50	-
Volume [m³]	57.10	5,000.00	-
Mat Density FW [kg/m²]	-	1.00	-
Stocking Density FW [kg/m³]	48.32	-	-
DM Content [%]	22.00	5.00	22.00
Standing Biomass DM [t]	0.61	0.50	-
Standing Biomass FW [t]	2.76	10.00	-
Feeding Rate [t FW/t FW/d]	1.00		
SGR [%]	1.03	27.40	-
FCR [-]	4.95	-	-
Annual DM Output [t]	2.29	50.00	1.53
Annual FW Output [t]	10.41	1,000.00	6.97

Table 6.8: Concentration of elements in mg/kg on a DM basis for the whole body, fillet and carcass of Nile tilapia, after [Gonzales and Brown, 2006].

Element	**Whole body [mg/kg DM]**	**Fillet [mg/kg DM]**	**Carcass [mg/kg DM]**
Ca	4,761.50	297.75	6,495.65
P	258.70	27.19	348.66
Mg	127.50	154.15	116.95
K	56.85	140.40	24.66
Na	394.75	7.36	545.21
Fe	0.33	3.39	0.00
Cu	0.53	0.02	0.73
Mn	0.26	1.37	0.00
Zn	13.45	0.57	18.45
B	0.38	0.68	0.27
Se	7.05	0.14	9.74
Mo	5.72	0.15	7.88
Co	0.62	0.00	0.85
Cr	0.07	0.03	0.09

As can be seen in table 6.8, the concentration of most essential elements is far higher in the carcass than in the fillet. By recycling the carcass back into the system, the loss of major plant nutrients and essential trace elements is greatly reduced.
Alternatively, the fish can be dressed (gutted) and sold, so only the guts would be recycled instead of the whole carcass.

Food Waste Recycling
Food waste of restaurants, groceries, cafeterias etc. can be fed to the fish in addition to duckweed. The amount fed should be in accordance with the amount of fish harvested on a DM basis. More than that could overload the system with nutrients. This strategy would provide the big majority of nutrients, sparing most fertilizer needs. Additionally, it would likely improve growth characteristics of the fish and total fish yield, as shown in table 6.9.
Food waste is available at very low costs, but comes with a significant disadvantage: it is potentially contaminated with preservatives, pesticides, packaging material, additives etc. The impact on the nutritional quality of the fish will be low, as more than 95 % of the diet will still be duckweed (DM basis), but it does not come with the same purity as fish fed 100 % out of the system. This might offset a very unique selling point.

Table 6.9: Parameters of the duckweed based aquaculture set-up with fish slaughter waste recycling

	Fish Cages	**Duckweed Pond**	**Food Waste Recycling**
Organism	Nile tilapia	Duckweed	-
Area [m²]	114.20	10,000.00	-
Depth [m]	0.5	0.50	-
Volume [m³]	57.10	5,000.00	-
Mat Density FW [kg/m²]	-	1.00	-
Stocking Density FW [kg/m³]	48.62	-	-
DM Content [%]	22.00	5.00	18.00
Standing Biomass DM [t]	0.61	0.50	-
Standing Biomass FW [t]	2.78	10.00	-
SGR [%]	1.08	27.40	-
FCR [-]	4.80	-	-
Annual DM Output [t]	2.40	50.00	2.4
Annual FW Output [t]	10.92	1,000.00	13.33

6.6 System Adoptions for Colder Climates

While this system is meant to be implemented in sub-tropical/tropical climates, it can easily be adjusted to temperate climates by switching from Nile tilapia to grass carp. The latter are adapted to colder climates and can overwinter in frozen lakes at a water temperature of 4°C.
The speed of the metabolism of the fish is directly depending on the water temperature, as is the growth rate of the floating plants. When duckweed is not growing in the winter, the grass carp do not need any feed.
All system parameters outlined in this case study can be transferred, except the annual productivities. In the months with a water surface temperature of around 17°C or more, the same growth rates can be assumed for duckweed. So, with a temperate climate where surface water temperatures get over 17°C in 26 weeks out of 52, the annual production is cut in half, as is the fish production.

6.7 Weaknesses and Strengths

Weaknesses
The great majority of global agriculture is soil based. Transforming land into pond area is an elaborate process that requires considerably more effort than preparing land for growing conventional crops. There also has to be water available year round.
The proposed system is complex as the three main components water, duckweed and fish are influencing each other and need to be kept in an equilibrium to maintain productivity. The system is depending on frequent close observation and measurements, the fish need to be fed daily and the harvest of the fish needs to be adjusted according to the current availability of duckweed. Therefore,the required skill sets for agricultural labourers are higher, the training takes longer.
The soil needs to have the right physical properties in order to hold the water, comparable to paddy rice cultivation. Soil with high water infiltration rates like sand will likely need additional liner material to hold the water.

The whole described set-up includes a number of assumptions due to lack of available data. The most important biological parameters are the duckweed productivity, the FCR and SGR of the fish. For instance, if the actual FCR is not at five, but at seven, the annual production of fish would drop from 10 t to 7.14 t (FW). More data needs to be gathered for more precise calculations regarding productivity and profitability. The closest system to the one proposed is that described by Skillicorn et al. (1992), which relies heavily on phytoplankton as a feed source. In this proposed system, phytoplankton has almost no role to play, making the transfer of any parameters difficult.
It is also unclear, how to harvest the duckweed and feed it to the fish in the most economical way. Delivering it by using pumps would be an elegant way, but this needs to be tested in practice first. Therefore, exact costs of labour can hardly be assessed.

Strengths
Duckweed ponds can be constructed on land otherwise unfit for crop production. Waterlogged soils and even swampland are especially suited for ponds. The most common issues of soil degradation like compaction, acidification and salinization are not problematic for duckweed cultivation. Hence, competition for arable land can be circumvented.
With an estimated annual yield of 10 t/ha of fish (FW) the system is superior to conventional livestock production. For instance the world's most efficient cattle grazing systems achieve 1.5 t/ha of live weight gain (FW) per year (see chapter 3.4.2). In aquaculture, protein yields are generally higher, especially in phytoplankton-based systems that are in the same order of productivity as duckweed-based systems.
However, the nitrogen efficiency for the open pond cultivation of duckweed is much higher than for phytoplankton due to a stable pH value of the water column during day and night.
The integration of the fish inside of the duckweed pond acting as a bio filter, enables direct nutrient absorption from the fish manure, so that any risk of pollution is minimized. The majority of nutrients stay inside of a self-recycling system, where only the harvest constitutes a removal of nutrients from the system. Provided the ponds are not leaking or overflowing, there is no pollution, no water erosion and no unwanted loss of nutrients.
The whole system is operated without any machinery besides solar-powered pumps. Once established, there is no sowing, ploughing, spraying fertilizer or pesticides, cutting or weeding involved.
High quality fish can be produced without the use of any imported feed such as soybean meal, fish meal or grain products. As the fish rely on green plant matter as main part of their diet, they will have a balanced ω-6/ω-3 ratio unlike grain-fed livestock products.

6.8 Comparison to Conventional Nile Tilapia Production

The following points are based on [Rakocy, 2009].
Several systems exist for the commercial production of Nile tilapia. In "low input" pond cultures, inorganic and/or organic fertilizers such as manure are used to facilitate the growth of phytoplankton as the feed source for the fish. For instance, reportedly, in Thailand, the pond cultures are supplied with 10.4 - 13.0 t DM/ha of chicken manure, urea fertilizer at 1.456 t N/ha and triple super phosphate fertilizer at 364 kg P/ha per year, resulting in annual fish yields of 8 - 11 t/ha (FW). Often times, pond cultures with high phytoplankton production are supplemented with feed, such as agricultural by-products like bran, oil-cakes and vegetation. With additional feeding, annual fish yields can be as high as 21 t/ha (FW).

In intensive production systems, farmers rely on high quality feeds based on soy and fish meal, realizing annual fish yields of 15 - 24 t/ha (FW). Continuous aeration is common in these types of systems, as well as water exchanges from five up to 150 % of the total water volume daily.
Intensive systems with a focus on higher quality inputs are predominantly producing for export, while pond cultures based on low quality inputs like manures are mainly producing for the local market. Off-flavours in the fish are only considered an issue for export produce.
In raceway ponds, the stocking density is up to 185 kg/m^3, while water exchange rate can be up to 180 complete volume changes per day in order to flush out nutrients, especially ammonia.
Fish tanks with low water exchange rates rely heavily on nitrification for the same purpose.
Recirculation systems are based on "a solids removal device, a biofilter, an aerator or oxygen generator and a degassing unit". These systems typically have water exchange rates of 5 - 10 % per day.
In tropical and subtropical countries, production costs can be as low as 0.55 - 0.65 US$/kg.
Comparing the conventional systems to the duckweed-based system proposed here, the main difference is the resource utilization. While the intensive systems have very high production rates, commercial feed needs to be imported, while the nutrient rich water gets discharged contributing to environmental pollution.
In the pond culture systems, nutrients are recycled, but the high-density phytoplankton culture is causing low nitrogen use efficiency, so that, again using the example of Thailand, about 2.2 t N/ha are used per year to compensate for the nitrogen loss through the pH increase caused by algal photosynthesis [Shammas et al., 2009].
Yields in the proposed duckweed-based system are expected to be similar to the yields in conventional pond culture, but at considerably lower nutrient inputs. Off-flavours in fish are also less likely, due to cleaner water and no reliance on phytoplankton, detritus and bacteria as the main feed source of the fish.

6.9 Conclusion

This case study was prepared to illustrate the feasibility of a duckweed-based aquaculture. While it can compete with conventional tilapia production systems, its clear unique selling point lies in its resource efficiency and clean production line.
Intensive systems require high inputs of high quality feeds often based on fish meal, while effluents are discharged without any nutrient recycling or treatment.
Low-tech pond cultures are more efficient in terms of nutrient recycling through phytoplankton growth, but usually produce a quality that doesn't meet the requirements for export. As the fish are kept in water very high in nutrients and organic matter, the fish can develop off-flavours or a "muddy" taste, so they can only be sold locally in developing countries.
Several options exist to modify the duckweed-based tilapia culture in order to adjust it to local needs. For instance, it can be extended by incorporating *Azolla* to be 100 % nitrogen independent.
While the proposed system needs to be evaluated in a pilot plant first in order to confirm or correct the calculated production parameters, it can certainly compete with the great majority of livestock production in terms of protein productivity and resource efficiency.

7. Summary and Conclusion

The contribution of global livestock production to the global diet is only modest at best in comparison to its resource consumption and environmental impact. Efforts in the last decades to increase the productivity of livestock production through measures of intensification have led to considerable advances in animal feeding efficiency through different choice of feed ingredients and selective breeding in crops and livestock.
However, these practices have vastly increased the competition for arable land, as more grain and oil crops are being fed to animals. While the productivity of livestock farming has gone up, global soil degradation is still accelerating, at alarming rates already decades ago.
The appeal to reduce animal product consumption for sustainability reasons seems appropriate, but does not tackle the actual root cause: wrong management practices on a global scale. Stopping all livestock farming today would greatly reduce pressure on agricultural resources, but only postpone the food supply shortfall that will eventually be the result of widespread soil degradation.

Farming systems with the capacity to feed future populations are available. They do

Table 7.1: Comparison of floating plant production with conventional feed crop production .

	Floating Plant Production	**Conventional Feed Crop Production**
Initial Land Preparation Effort	high	low
Competition for Arable Land	very low	very high
Impact on Soil Degradation	low	high
Dependence of Productivity on Soil Quality	very low	high
Protein and Biomass Productivity	very high	medium
Required Labour	high	low
Developed Market	no	yes
Specialized Equipment Available	no	yes
Effort of Drying the Harvest	very high	low - medium
Fertilizer Efficiency	medium - very high	low - medium
Pollution Potential	low	high
Water Requirement per Area	very high	medium
Water Requirement per Yield	low	medium
Suitability as Animal Feed	medium	high

not exclude animal husbandry, but integrate it in a way that makes use of synergies. Yield increases do not need to come at the expense of environmental protection. Also, feeding a growing world population does not mean that food quality needs to decline. It

is possible to get a higher yield and better nutritional quality at larger scales with less environmental pollution.
This thesis provides a conceptional framework for achieving a protein production for the livestock sector that does not compete for crop land, produces much higher yields per area, with minimized environmental pollution. Floating plant-based systems provide the possibility of increasing the resource efficiency of livestock production. The main differences between floating plant production and conventional feed production is shown in table 7.1.
The fact that more protein in the form of fish can be produced than on the same area of a state-of-the-art soybean field makes one wonder about the efficiency of modern agriculture.
By better understanding the interactions of animals and plants, farming systems can be developed and implemented that are capable of securing food supply for future generations.
All the conducted experiments showed that floating plants were capable of replacing part of the diet of poultry and had a positive impact on the fatty acid profiles of the eggs. The effect on laying performance and feed efficiency was somewhat inconclusive. There was a clear tendency towards duckweed being able to constitute a bigger part of the diet of poultry than *Azolla*. The experiment with the grass carp showed good growth performance with duckweed as sole feed source, compared to the literature that is very focused on fingerlings.
The cultivation of *Azolla* showed very high biomass productivity, while duckweed showed only modest growth rates, compared to the literature. The main reason for this was low nitrogen concentrations in the water and devastating insect infestations on both plants.
The case study on a duckweed-based tilapia production system proved to be competitive, based on available production parameters and has a high potential for the local production of animal protein with minimum pollution potential.
Duckweed pond culture also enables the local recycling of liquid manure. The disposal thereof becomes an increasing problem in areas with a high density of livestock, while nitrate concentrations in the ground water are rising accordingly. Duckweed can take up more nitrogen per area than conventional crops under optimal conditions and could therefore improve local nutrient recycling and water protection.

Appendices

Appendix A

Table A.1: Land allocation based on FAO data.

Annual population		7700000000		FAOSTAT, Population, Annual Population, World + (Total), Total Population - Both sexes, 201
	%	1000 ha	m^2 per capita	Source
Land area	100.0	12994900.2	1687649371	FAOSTAT, Inputs, Land Use, World + (Total), Area, 2015
Agricultural land	36.9	4797557.3	623059393	FAOSTAT, Inputs, Land Use, World + (Total), Area, 2015
Forest land	30.7	3999133.6	519368003	FAOSTAT, Inputs, Land Use, World + (Total), Area, 2015
Terrestrial barren land	14.5	1883997.3	244674971	FAOSTAT, Agri-Environmental Indicators, Land Cover, World + (Total), CCI_LC, 2015
Shrub-covered areas	12.5	1627341.7	211343074	FAOSTAT, Agri-Environmental Indicators, Land Cover, World + (Total), CCI_LC, 2015
Inland waters	3.5	459315.6	59651381	FAOSTAT, Inputs, Land Use, World + (Total), Area, 2015
Permanent snow and glaciers	0.6	84292.9	10947131	FAOSTAT, Agri-Environmental Indicators, Land Cover, World + (Total), CCI_LC, 2015
Artificial surfaces (including urban and associated area	0.4	55399.0	7194670	FAOSTAT, Agri-Environmental Indicators, Land Cover, World + (Total), CCI_LC, 2015
Rest	0.8	87862.8	11410749	
Land under perm. meadows and pastures	66.7	3247027.34	421691863	FAOSTAT, Inputs, Land Use, World + (Total), Area, 2015
Land under permanent crops	3.4	165298.304	21467312	FAOSTAT, Inputs, Land Use, World + (Total), Area, 2015
Temporary cropland	28.4	1385228.88	179899855	Cropland - Land under permanent crops
Land under permanent crops		165298.304	21467312	FAOSTAT, Inputs, Land Use, World + (Total), Area, 2015
Cropland		1550527.18	201367167	FAOSTAT, Inputs, Land Use, World + (Total), Area, 2015
Arable land for feed production		512534.686	66562946	Arable land*0.37
Arable land for food crops		872694.195	113336908	Arable land*0.63
Agricultural land for feed production		3759562.03	488254809	Land under perm. meadows and pastures + Arable land for feed production
Agricultural land for food crops		1037992.5	134804221	Arable land for food crops + Land under permanent crops
Wheat	15.3	218543.071	28382217	FAOSTAT, Production, Crops, World + (Total), Area, 2017
Maize	13.8	197185.936	25608563	FAOSTAT, Production, Crops, World + (Total), Area, 2017
Rice, paddy	11.7	167249.103	21720663	FAOSTAT, Production, Crops, World + (Total), Area, 2017
Soybeans	8.7	123551.146	16045603	FAOSTAT, Production, Crops, World + (Total), Area, 2017
Barley	3.3	47009.175	6105088	FAOSTAT, Production, Crops, World + (Total), Area, 2017
Sorghum	2.9	40674.113	5282352	FAOSTAT, Production, Crops, World + (Total), Area, 2017
Beans, dry	2.6	36458.894	4734921	FAOSTAT, Production, Crops, World + (Total), Area, 2017
Rapeseed	2.4	34740.403	4511741	FAOSTAT, Production, Crops, World + (Total), Area, 2017
Millet	2.2	31244.432	4057718	FAOSTAT, Production, Crops, World + (Total), Area, 2017
Sunflower	1.9	26533.596	3445922	FAOSTAT, Production, Crops, World + (Total), Area, 2017
Remaining temporary crops	32.4	462039.012	60005066	Temporary cropland - Sum of Top 10 Crops

Table A.2: Calculation of global animal protein production based on FAO data.

Animal product production 2013	[t/y]	[t Protein/y]	[million kcal/y]	[g/capita/day]	[g Protein/capita/day]	[g Protein/g]	[kcal/capita/day]	[kcal/g]
Animal fats	37474811	329448.89	251204777.03	9.1	0.08	0.008791209	61	6.703296703
Aquatic Products, Other	25198308	703401.04	8275306.40	6.09	0.17	0.027914614	2	0.328407225
Eggs	73785370	8172337.53	105449516.47	25.19	2.79	0.110758237	36	1.429138547
Fish, Seafood	154848221	15541390.38	101227446.91	52.01	5.22	0.100365314	34	0.653720438
Meat	309121367	37958151.14	618712642.34	118.41	14.54	0.122793683	237	2.001520142
Milk - Excluding Butter	753035981	25104253.41	421458268.96	246.57	8.22	0.033337389	138	0.559678793
Offals	18592398	3330885.64	21196544.95	6.14	1.1	0.179153094	7	1.140065147
Source or Calculation	FAOSTAT, Commodity Balances - Livestock and Fish Primary Equivalent, World + (Total), Production Quantity, 2013	[t/y]*[g Protein/capita/day]	[t/y]*[kcal/g]	FAOSTAT, Food Supply - Livestock and Fish Primary Equivalent, World + (Total), Food supply quantity (g/capita/day),	FAOSTAT, Food Supply - Livestock and Fish Primary Equivalent, World + (Total), Protein supply quantity (g/capita/day), 2013	[g Protein/capita/day]/[g/capita/day]	FAOSTAT, Food Supply - Livestock and Fish Primary Equivalent, World + (Total), Food supply (g/capita/day),	[kcal/capita/day]/[g/capita/day]
Total Animal Products	1372056456	91139868.02	1527524503	463.51	32.12			

Appendix B

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ilac-MRA DAkkS Deutsche Akkreditierungsstelle D-PL-14165-01-00

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Dr. Michael Egert
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Prüfbericht

Oldenburg, 20.06.2019
Seite 1 von 1
Berichts-Version: 1

Kunden-Nr.:	50178422	Probeneingang:	04.06.2019
Auftrags-Nr.:	1649882	Untersuchungsbeginn:	04.06.2019
Proben-Nr.:	**19FU023249**	Untersuchungsende:	20.06.2019
Probenart:	Weizen		
Befindlich in:	Siegeltüte, Plombe/Siegel: 84703482		
Bezeichnung:	Weizen		
Probenehmer:	durch Auftraggeber		

	Ergebnis in der Originalsubstanz
Wasser *Methode: VO (EG) 152 Anhang III, A; 2009*	**13,9 %**
Rohasche *Methode: VO (EG) 152 Anhang III, M; 2009*	**1,6 %**
Rohprotein *Methode: VO (EG) 152 Anhang III, C; 2009*	**10,7 %**
Rohfett A+B *Methode: VO (EG) 152 Anhang III, H; 2009*	**2,5 %**
Stärke *Methode: VO (EG) 152 Anhang III, L; 2009*	**57,4 %**
Gesamtzucker ber. als Saccharose *Methode: VO (EG) 152 Anhang III, J; 2009*	**2,3 %**
Fettsäuremuster *Methode: ASU L 13.00-26; 2008-06 / ASU L 13.00-27/2; 2012-01*	**siehe Anlage**

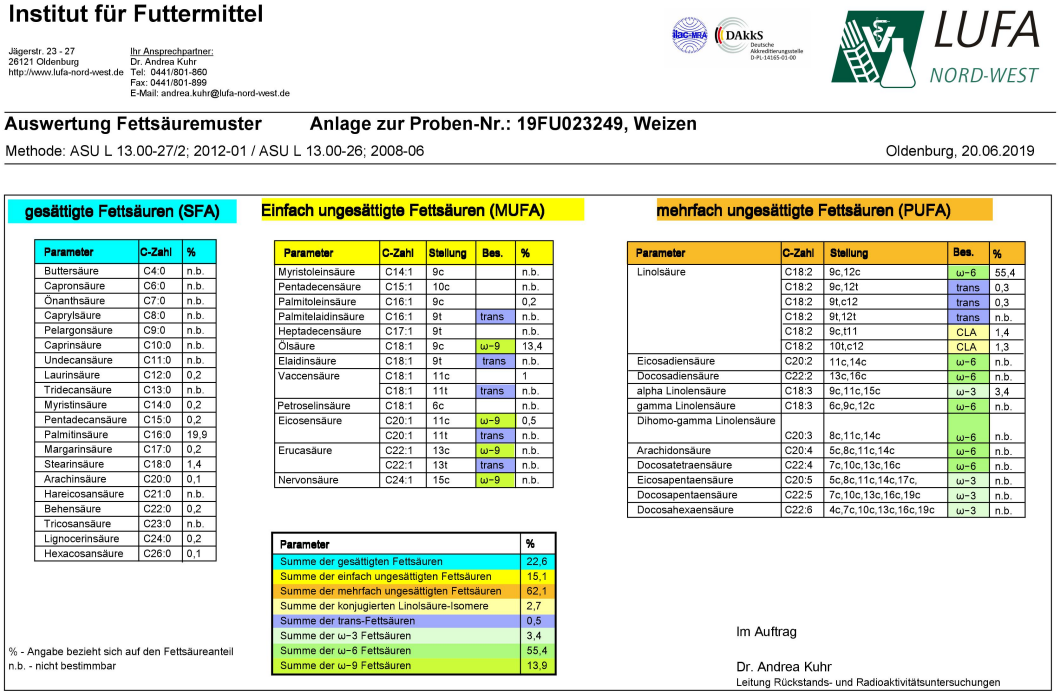
Im Auftrag

Dr. Michael Egert
Institutsleiter

Dieser Prüfbericht wurde maschinell erstellt und ist ohne Unterschrift gültig.

#2 = IfT, Oldenburg; **#3** = IfL, Oldenburg; **#4** = IfB, IfD, Hameln; **#5** = Untersuchung erfolgte in Fremdlabor; **#6** = unterliegt nicht der Akkreditierung
„<…" = Wert ist kleiner als die nebenstehende untere Grenze des Arbeitsbereiches

Figure B.1: Lab report of nutritional analysis of wheat grains.



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LUFA NORD-WEST

Auswertung Fettsäuremuster **Anlage zur Proben-Nr.: 19FU023249, Weizen**

Methode: ASU L 13.00-27/2; 2012-01 / ASU L 13.00-26; 2008-06 Oldenburg, 20.06.2019

gesättigte Fettsäuren (SFA)

Parameter	C-Zahl	%
Buttersäure	C4:0	n.b.
Capronsäure	C6:0	n.b.
Önanthsäure	C7:0	n.b.
Caprylsäure	C8:0	n.b.
Pelargonsäure	C9:0	n.b.
Caprinsäure	C10:0	n.b.
Undecansäure	C11:0	n.b.
Laurinsäure	C12:0	0,2
Tridecansäure	C13:0	n.b.
Myristinsäure	C14:0	0,2
Pentadecansäure	C15:0	0,2
Palmitinsäure	C16:0	19,9
Margarinsäure	C17:0	0,2
Stearinsäure	C18:0	1,4
Arachinsäure	C20:0	0,1
Hareicosansäure	C21:0	n.b.
Behensäure	C22:0	0,2
Tricosansäure	C23:0	n.b.
Lignocerinsäure	C24:0	0,2
Hexacosansäure	C26:0	0,1

Einfach ungesättigte Fettsäuren (MUFA)

Parameter	C-Zahl	Stellung	Bes.	%
Myristoleinsäure	C14:1	9c		n.b.
Pentadecensäure	C15:1	10c		n.b.
Palmitoleinsäure	C16:1	9c		0,2
Palmitelaidinsäure	C16:1	9t	trans	n.b.
Heptadecensäure	C17:1	9t		n.b.
Ölsäure	C18:1	9c	ω−9	13,4
Elaidinsäure	C18:1	9t	trans	n.b.
Vaccensäure	C18:1	11c		1
	C18:1	11t	trans	n.b.
Petroselinsäure	C18:1	6c		n.b.
Eicosensäure	C20:1	11c	ω−9	0,5
	C20:1	11t	trans	n.b.
Erucasäure	C22:1	13c	ω−9	n.b.
	C22:1	13t	trans	n.b.
Nervonsäure	C24:1	15c	ω−9	n.b.

mehrfach ungesättigte Fettsäuren (PUFA)

Parameter	C-Zahl	Stellung	Bes.	%
Linolsäure	C18:2	9c,12c	ω−6	55,4
	C18:2	9c,12t	trans	0,3
	C18:2	9t,c12	trans	0,3
	C18:2	9t,12t	trans	n.b.
	C18:2	9c,t11	CLA	1,4
	C18:2	10t,c12	CLA	1,3
Eicosadiensäure	C20:2	11c,14c	ω−6	n.b.
Docosadiensäure	C22:2	13c,16c	ω−6	n.b.
alpha Linolensäure	C18:3	9c,11c,15c	ω−3	3,4
gamma Linolensäure	C18:3	6c,9c,12c	ω−6	n.b.
Dihomo-gamma Linolensäure	C20:3	8c,11c,14c	ω−6	n.b.
Arachidonsäure	C20:4	5c,8c,11c,14c	ω−6	n.b.
Docosatetraensäure	C22:4	7c,10c,13c,16c	ω−6	n.b.
Eicosapentaensäure	C20:5	5c,8c,11c,14c,17c,	ω−3	n.b.
Docosapentaensäure	C22:5	7c,10c,13c,16c,19c	ω−3	n.b.
Docosahexaensäure	C22:6	4c,7c,10c,13c,16c,19c	ω−3	n.b.

Parameter	%
Summe der gesättigten Fettsäuren	22,6
Summe der einfach ungesättigten Fettsäuren	15,1
Summe der mehrfach ungesättigten Fettsäuren	62,1
Summe der konjugierten Linolsäure-Isomere	2,7
Summe der trans-Fettsäuren	0,5
Summe der ω−3 Fettsäuren	3,4
Summe der ω−6 Fettsäuren	55,4
Summe der ω−9 Fettsäuren	13,9

% - Angabe bezieht sich auf den Fettsäureanteil
n.b. - nicht bestimmbar

Im Auftrag

Dr. Andrea Kuhr
Leitung Rückstands- und Radioaktivitätsuntersuchungen

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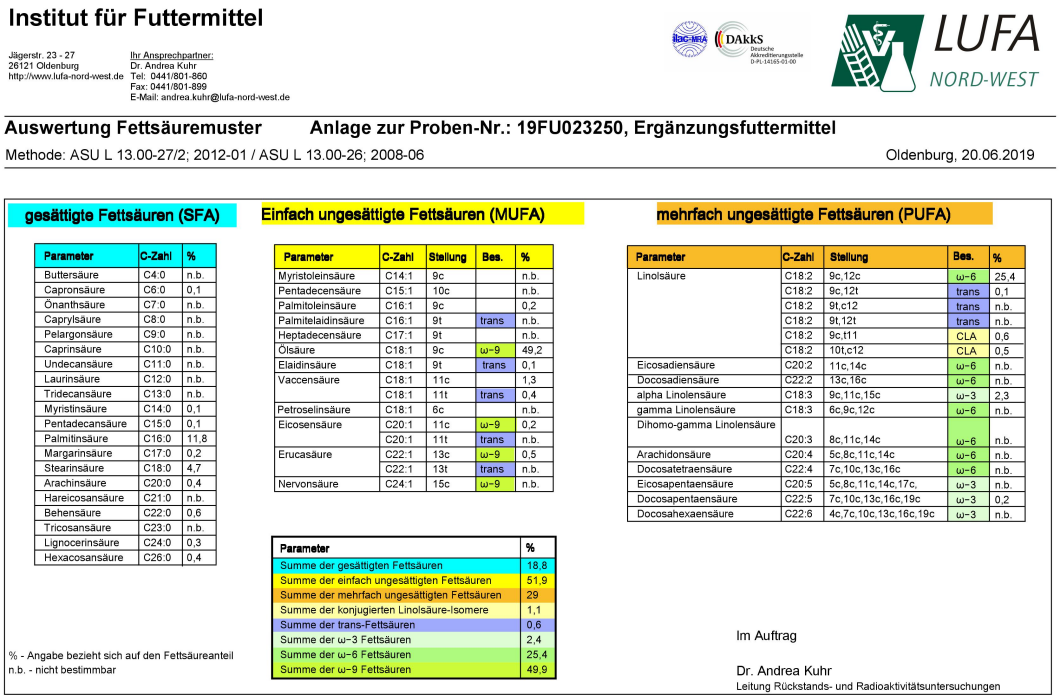
Figure B.2: Lab report of the FA profile of the wheat grains.



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LUFA NORD-WEST

Auswertung Fettsäuremuster **Anlage zur Proben-Nr.: 19FU023250, Ergänzungsfuttermittel**

Methode: ASU L 13.00-27/2; 2012-01 / ASU L 13.00-26; 2008-06 Oldenburg, 20.06.2019

gesättigte Fettsäuren (SFA)

Parameter	C-Zahl	%
Buttersäure	C4:0	n.b.
Capronsäure	C6:0	0,1
Önanthsäure	C7:0	n.b.
Caprylsäure	C8:0	n.b.
Pelargonsäure	C9:0	n.b.
Caprinsäure	C10:0	n.b.
Undecansäure	C11:0	n.b.
Laurinsäure	C12:0	n.b.
Tridecansäure	C13:0	n.b.
Myristinsäure	C14:0	0,1
Pentadecansäure	C15:0	0,1
Palmitinsäure	C16:0	11,8
Margarinsäure	C17:0	0,2
Stearinsäure	C18:0	4,7
Arachinsäure	C20:0	0,4
Hareicosansäure	C21:0	n.b.
Behensäure	C22:0	0,6
Tricosansäure	C23:0	n.b.
Lignocerinsäure	C24:0	0,3
Hexacosansäure	C26:0	0,4

Einfach ungesättigte Fettsäuren (MUFA)

Parameter	C-Zahl	Stellung	Bes.	%
Myristoleinsäure	C14:1	9c		n.b.
Pentadecensäure	C15:1	10c		n.b.
Palmitoleinsäure	C16:1	9c		0,2
Palmitelaidinsäure	C16:1	9t	trans	n.b.
Heptadecensäure	C17:1	9t		n.b.
Ölsäure	C18:1	9c	ω−9	49,2
Elaidinsäure	C18:1	9t	trans	0,1
Vaccensäure	C18:1	11c		1,3
	C18:1	11t	trans	0,4
Petroselinsäure	C18:1	6c		n.b.
Eicosensäure	C20:1	11c	ω−9	0,2
	C20:1	11t	trans	n.b.
Erucasäure	C22:1	13c	ω−9	0,5
	C22:1	13t	trans	n.b.
Nervonsäure	C24:1	15c	ω−9	n.b.

mehrfach ungesättigte Fettsäuren (PUFA)

Parameter	C-Zahl	Stellung	Bes.	%
Linolsäure	C18:2	9c,12c	ω−6	25,4
	C18:2	9c,12t	trans	0,1
	C18:2	9t,c12	trans	n.b.
	C18:2	9t,12t	trans	n.b.
	C18:2	9c,t11	CLA	0,6
	C18:2	10t,c12	CLA	0,5
Eicosadiensäure	C20:2	11c,14c	ω−6	n.b.
Docosadiensäure	C22:2	13c,16c	ω−6	n.b.
alpha Linolensäure	C18:3	9c,11c,15c	ω−3	2,3
gamma Linolensäure	C18:3	6c,9c,12c	ω−6	n.b.
Dihomo-gamma Linolensäure	C20:3	8c,11c,14c	ω−6	n.b.
Arachidonsäure	C20:4	5c,8c,11c,14c	ω−6	n.b.
Docosatetraensäure	C22:4	7c,10c,13c,16c	ω−6	n.b.
Eicosapentaensäure	C20:5	5c,8c,11c,14c,17c,	ω−3	n.b.
Docosapentaensäure	C22:5	7c,10c,13c,16c,19c	ω−3	0,2
Docosahexaensäure	C22:6	4c,7c,10c,13c,16c,19c	ω−3	n.b.

Parameter	%
Summe der gesättigten Fettsäuren	18,8
Summe der einfach ungesättigten Fettsäuren	51,9
Summe der mehrfach ungesättigten Fettsäuren	29
Summe der konjugierten Linolsäure-Isomere	1,1
Summe der trans-Fettsäuren	0,6
Summe der ω−3 Fettsäuren	2,4
Summe der ω−6 Fettsäuren	25,4
Summe der ω−9 Fettsäuren	49,9

% - Angabe bezieht sich auf den Fettsäureanteil
n.b. - nicht bestimmbar

Im Auftrag

Dr. Andrea Kuhr
Leitung Rückstands- und Radioaktivitätsuntersuchungen

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Figure B.3: Lab report of the FA profile of the supplementary concentrate

Legehennen Ergänzer LE 50

Ergänzungsfuttermittel für Legehennen

Zulassungsnummer: αDE-BY-1-00020

Gehalt an Inhaltsstoffen:

28,10 %	Rohprotein	0,55 %	Methionin
9,50 %	Rohöle und -fette	6,70 %	Calcium
10,20 %	Rohfaser	0,97 %	Phosphor
24,50 %	Rohasche	0,33 %	Natrium
1,08 %	Lysin	8,80MJ	ME/kg

Zusammensetzung:

Sojakuchen a.d.Umstellung, A-Bio Sonnenblumenkuchen, Calciumcarbonat, Maiskleber, A-Bio Leinkuchen, A-Bio Grünmehl, A-Bio Sesamkuchen, A-Bio Erbsen, Monocalciumphosphat, A-Bio Sojaöl

Gehalt an Zusatzstoffen je kg:

Ernährungsphysiologische Zusatzstoffe:

19.200 I.E.	Vitamin A (3a672a)
5.200 I.E.	Vitamin D3 (3a671)
115,00 mg	Vitamin E (3a700)
180,00 mg	Zink als Zinkoxid (3b603)
135,00 mg	Eisen als Eisen(II)-sulfat, Monohydrat (3b103)
180,00 mg	Mangan als Mangan(II)-oxid (3b502)
24,00 mg	Kupfer als Kupfer-(II)-sulfat, Pentahydrat (E4)
0,90 mg	Selen als Natriumselenit (E 8)

Technologische Zusatzstoffe:
Ameisensäure (E236) , Milchsäure (E270)

Fütterungshinweis:

Mischungsbeispiel:

bis ca. 20. Legewoche:	ab ca. 20. Legewoche:
50% Ergänzer	50% Ergänzer
45% Getreide (Weizen/Mais)	50% Getreide (Weizen/Mais)
5% Erbsen	

Ab der ca. 20. Legewoche zusätzlich Muschelschalen zur freien Aufnahme anbieten.

Nettomasse: 25 kg oder Wiegeschein
Chargen Nr: 904350

Artikel Nr: 9400500 / 7
Stand: 20.08.2018

4 Monate vor dem angegebenen Mindesthaltbarkeitsdatum hergestellt. Einschließlich Zusatzstoffe mindestens haltbar bis:

MEIKA ÖKOLOGISCHE TIERERNÄHRUNG

Meika Tierernährung GmbH
86845 Großaitingen, Bahnhofstr. 95-99
Tel.: 08203/96080 Fax: 08203/951986

EG Kontollnummer: DE-BY-006-47032-CDE
Codenummer: DE-ÖKO-006
KAT ID Nr.: D-86845-1

Kann in der biologischen Produktion gem. den Verordnungen (EG) Nr. 834/2007 und (EG) Nr. 889/2008 verwendet werden. Entspricht den Bioland-/Naturland-/Biokreis-/Bio Austria - Richtlinien. Für die Nutzung des Warenzeichens ist ein Vertrag mit dem jeweiligen Verband erforderlich.

88 % TS, davon mineralischer Anteil und Zusatzstoffe 21,50% und landw. Ursprungs 78,50%, davon: aus biologischer Landwirtschaft 54,78%, aus Umstellung 33,76% und aus nicht biologischer Landwirtschaft 11,46%.

Unser Geflügelfutter wird risikoorientiert nach einem HACCP-System entsprechend den Vorgaben der FuttermittelhygieneVO (EG) 183/2005 auf Salmonellen untersucht.

Figure B.4: Manufacturer's specifications of the supplementary concentrate.

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Prüfbericht

Oldenburg, 20.06.2019
Seite 1 von 1
Berichts-Version: 1

Kunden-Nr.:	50178422	Probeneingang:	04.06.2019
Auftrags-Nr.:	1649882	Untersuchungsbeginn:	04.06.2019
Proben-Nr.:	**19FU023250**	Untersuchungsende:	20.06.2019
Probenart:	Ergänzungsfuttermittel		
Befindlich in:	Siegeltüte, Plombe/Siegel: 84703480		
Bezeichnung:	Ergänzungsfuttermittel		
Probenehmer:	durch Auftraggeber		

	Ergebnis in der Originalsubstanz
Wasser *Methode: VO (EG) 152 Anhang III, A; 2009*	**7,6 %**
Rohasche *Methode: VO (EG) 152 Anhang III, M; 2009*	**18,6 %**
Rohprotein *Methode: VO (EG) 152 Anhang III, C; 2009*	**29,0 %**
Rohfett A+B *Methode: VO (EG) 152 Anhang III, H; 2009*	**9,0 %**
Stärke *Methode: VO (EG) 152 Anhang III, L; 2009*	**3,7 %**
Gesamtzucker ber. als Saccharose *Methode: VO (EG) 152 Anhang III, J; 2009*	**4,1 %**
Fettsäuremuster *Methode: ASU L 13.00-26; 2008-06 / ASU L 13.00-27/2; 2012-01*	**siehe Anlage**

Im Auftrag

Dr. Michael Egert
Institutsleiter

Dieser Prüfbericht wurde maschinell erstellt und ist ohne Unterschrift gültig.

#2 = IfT, Oldenburg; #3 = IfL, Oldenburg; #4 = IfB, IfD, Hameln; #5 = Untersuchung erfolgte in Fremdlabor; #6 = unterliegt nicht der Akkreditierung
„<…" = Wert ist kleiner als die nebenstehende untere Grenze des Arbeitsbereiches

Figure B.5: Lab report of nutritional analysis of the supplementary concentrate.

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Prüfbericht

Oldenburg, 03.07.2019
Seite 1 von 1
Berichts-Version: 1

Kunden-Nr.:	50178422	Probeneingang:	04.06.2019
Auftrags-Nr.:	1649882	Untersuchungsbeginn:	04.06.2019
Proben-Nr.:	**19FU023251**	Untersuchungsende:	03.07.2019
Probenart:	Sonstige		
Befindlich in:	Siegeltüte, Plombe/Siegel: 84703472, 84703474, 84703484, 84703492, 84703494		
Bezeichnung:	Wasserlinsen		
Probenehmer:	durch Auftraggeber		

	Ergebnis in der Originalsubstanz	Berechnet auf 100 % Trockensubstanz
Wasser *Methode: VO (EG) 152 Anhang III, A; 2009*	**94,4 %**	
Rohasche *Methode: VO (EG) 152 Anhang III, M; 2009*	**0,9 %**	16,3 %
Rohprotein *Methode: VO (EG) 152 Anhang III, C; 2009*	**1,9 %**	33,7 %
Rohfett A+B *Methode: VO (EG) 152 Anhang III, H; 2009*	**0,5 %**	9,3 %
Stärke *Methode: VO (EG) 152 Anhang III, L; 2009*	**0,7 %**	12,4 %
Gesamtzucker ber. als Saccharose *Methode: VO (EG) 152 Anhang III, J; 2009*	**0,3 %**	5,4 %
Fettsäuremuster *Methode: ASU L 13.00-26; 2008-06 / ASU L 13.00-27/2; 2012-01*	**siehe Anlage**	

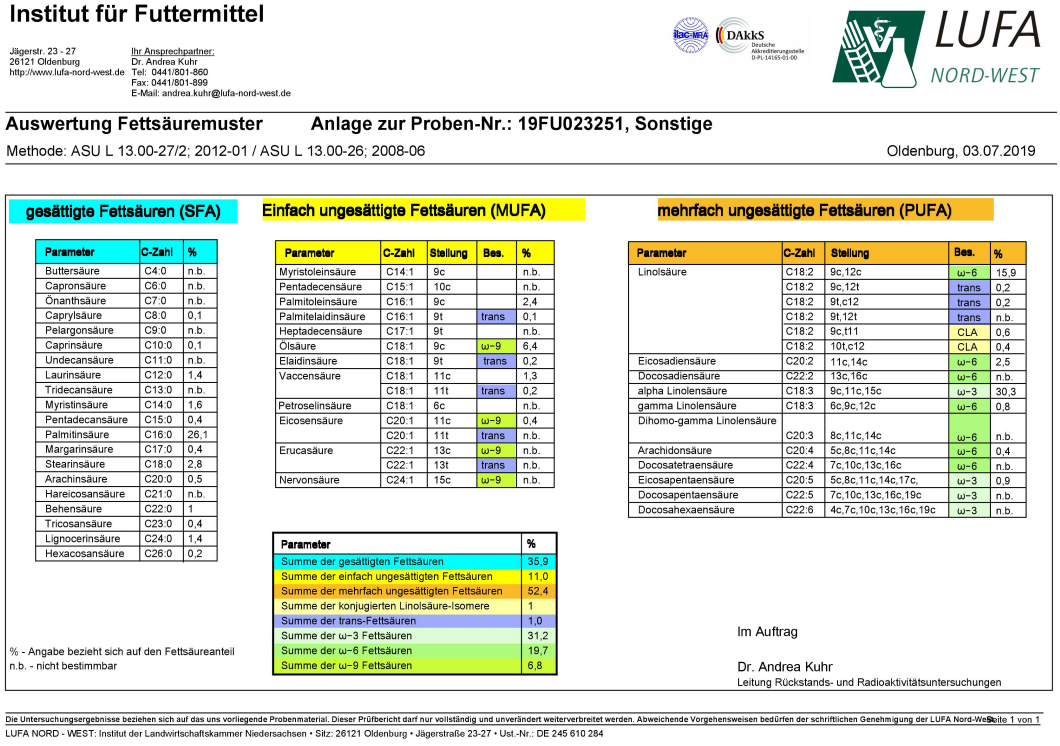
Im Auftrag

Dr. Hartwig Wellmann
Laborbereichsleiter

Dieser Prüfbericht wurde maschinell erstellt und ist ohne Unterschrift gültig.

#2 = IfT, Oldenburg; #3 = IfL, Oldenburg; #4 = IfB, IfD, Hameln; #5 = Untersuchung erfolgte in Fremdlabor; #6 = unterliegt nicht der Akkreditierung
„<…" = Wert ist kleiner als die nebenstehende untere Grenze des Arbeitsbereiches

Figure B.6: Lab report of the nutritional analysis of duckweed.



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LUFA NORD-WEST

Auswertung Fettsäuremuster **Anlage zur Proben-Nr.: 19FU023251, Sonstige**

Methode: ASU L 13.00-27/2; 2012-01 / ASU L 13.00-26; 2008-06 Oldenburg, 03.07.2019

gesättigte Fettsäuren (SFA)

Parameter	C-Zahl	%
Buttersäure	C4:0	n.b.
Capronsäure	C6:0	n.b.
Önanthsäure	C7:0	n.b.
Caprylsäure	C8:0	0,1
Pelargonsäure	C9:0	n.b.
Caprinsäure	C10:0	0,1
Undecansäure	C11:0	n.b.
Laurinsäure	C12:0	1,4
Tridecansäure	C13:0	n.b.
Myristinsäure	C14:0	1,6
Pentadecansäure	C15:0	0,4
Palmitinsäure	C16:0	26,1
Margarinsäure	C17:0	0,4
Stearinsäure	C18:0	2,8
Arachinsäure	C20:0	0,5
Hareicosansäure	C21:0	n.b.
Behensäure	C22:0	1
Tricosansäure	C23:0	0,4
Lignocerinsäure	C24:0	1,4
Hexacosansäure	C26:0	0,2

Einfach ungesättigte Fettsäuren (MUFA)

Parameter	C-Zahl	Stellung	Bes.	%
Myristoleinsäure	C14:1	9c		n.b.
Pentadecensäure	C15:1	10c		n.b.
Palmitoleinsäure	C16:1	9c		2,4
Palmitelaidinsäure	C16:1	9t	trans	0,1
Heptadecensäure	C17:1	9t		n.b.
Ölsäure	C18:1	9c	ω−9	6,4
Elaidinsäure	C18:1	9t	trans	0,2
Vaccensäure	C18:1	11c		1,3
	C18:1	11t	trans	0,2
Petroselinsäure	C18:1	6c		n.b.
Eicosensäure	C20:1	11c	ω−9	0,4
	C20:1	11t	trans	n.b.
Erucasäure	C22:1	13c	ω−9	n.b.
	C22:1	13t	trans	n.b.
Nervonsäure	C24:1	15c	ω−9	n.b.

mehrfach ungesättigte Fettsäuren (PUFA)

Parameter	C-Zahl	Stellung	Bes.	%
Linolsäure	C18:2	9c,12c	ω−6	15,9
	C18:2	9c,12t	trans	0,2
	C18:2	9t,c12	trans	0,2
	C18:2	9t,12t	trans	n.b.
	C18:2	9c,t11	CLA	0,6
	C18:2	10t,c12	CLA	0,4
Eicosadiensäure	C20:2	11c,14c	ω−6	2,5
Docosadiensäure	C22:2	13c,16c	ω−6	n.b.
alpha Linolensäure	C18:3	9c,11c,15c	ω−3	30,3
gamma Linolensäure	C18:3	6c,9c,12c	ω−6	0,8
Dihomo-gamma Linolensäure	C20:3	8c,11c,14c	ω−6	n.b.
Arachidonsäure	C20:4	5c,8c,11c,14c	ω−6	0,4
Docosatetraensäure	C22:4	7c,10c,13c,16c	ω−6	n.b.
Eicosapentaensäure	C20:5	5c,8c,11c,14c,17c,	ω−3	0,9
Docosapentaensäure	C22:5	7c,10c,13c,16c,19c	ω−3	n.b.
Docosahexaensäure	C22:6	4c,7c,10c,13c,16c,19c	ω−3	n.b.

Parameter	%
Summe der gesättigten Fettsäuren	35,9
Summe der einfach ungesättigten Fettsäuren	11,0
Summe der mehrfach ungesättigten Fettsäuren	52,4
Summe der konjugierten Linolsäure-Isomere	1
Summe der trans-Fettsäuren	1,0
Summe der ω−3 Fettsäuren	31,2
Summe der ω−6 Fettsäuren	19,7
Summe der ω−9 Fettsäuren	6,8

% - Angabe bezieht sich auf den Fettsäureanteil
n.b. - nicht bestimmbar

Im Auftrag

Dr. Andrea Kuhr
Leitung Rückstands- und Radioaktivitätsuntersuchungen

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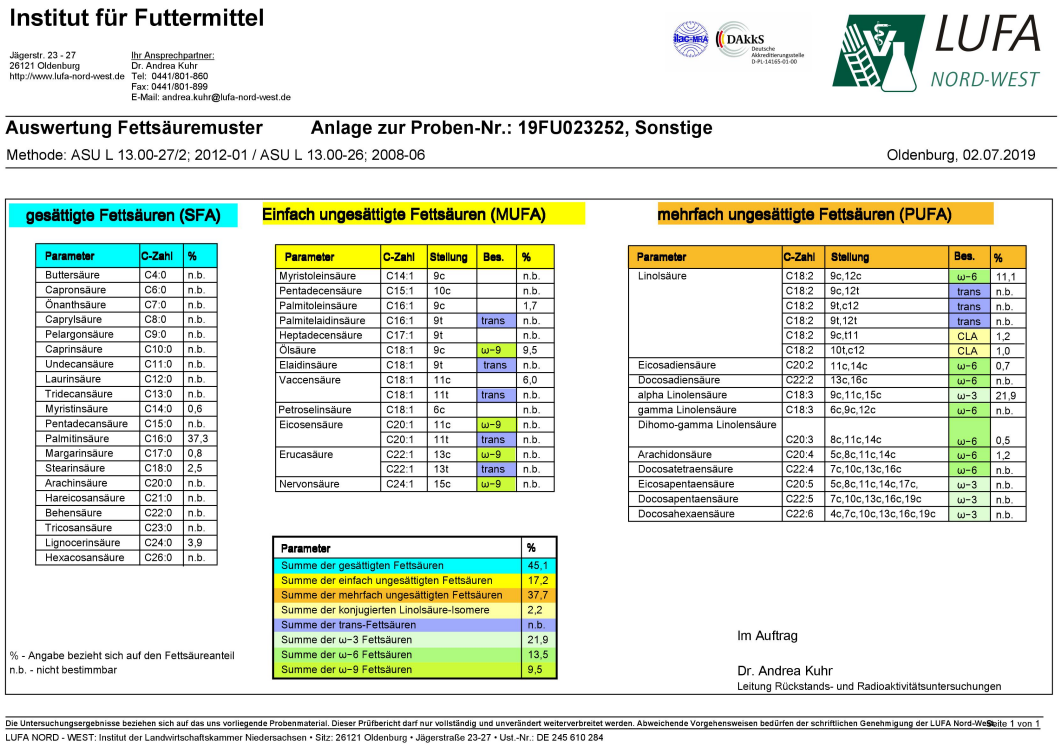
Figure B.7: Lab report of the FA profile of duckweed.



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LUFA NORD-WEST

Auswertung Fettsäuremuster **Anlage zur Proben-Nr.: 19FU023252, Sonstige**

Methode: ASU L 13.00-27/2; 2012-01 / ASU L 13.00-26; 2008-06 Oldenburg, 02.07.2019

gesättigte Fettsäuren (SFA)

Parameter	C-Zahl	%
Buttersäure	C4:0	n.b.
Capronsäure	C6:0	n.b.
Önanthsäure	C7:0	n.b.
Caprylsäure	C8:0	n.b.
Pelargonsäure	C9:0	n.b.
Caprinsäure	C10:0	n.b.
Undecansäure	C11:0	n.b.
Laurinsäure	C12:0	n.b.
Tridecansäure	C13:0	n.b.
Myristinsäure	C14:0	0,6
Pentadecansäure	C15:0	n.b.
Palmitinsäure	C16:0	37,3
Margarinsäure	C17:0	0,8
Stearinsäure	C18:0	2,5
Arachinsäure	C20:0	n.b.
Hareicosansäure	C21:0	n.b.
Behensäure	C22:0	n.b.
Tricosansäure	C23:0	n.b.
Lignocerinsäure	C24:0	3,9
Hexacosansäure	C26:0	n.b.

Einfach ungesättigte Fettsäuren (MUFA)

Parameter	C-Zahl	Stellung	Bes.	%
Myristoleinsäure	C14:1	9c		n.b.
Pentadecensäure	C15:1	10c		n.b.
Palmitoleinsäure	C16:1	9c		1,7
Palmitelaidinsäure	C16:1	9t	trans	n.b.
Heptadecensäure	C17:1	9t		n.b.
Ölsäure	C18:1	9c	ω−9	9,5
Elaidinsäure	C18:1	9t	trans	n.b.
Vaccensäure	C18:1	11c		6,0
	C18:1	11t	trans	n.b.
Petroselinsäure	C18:1	6c		n.b.
Eicosensäure	C20:1	11c	ω−9	n.b.
	C20:1	11t	trans	n.b.
Erucasäure	C22:1	13c	ω−9	n.b.
	C22:1	13t	trans	n.b.
Nervonsäure	C24:1	15c	ω−9	n.b.

mehrfach ungesättigte Fettsäuren (PUFA)

Parameter	C-Zahl	Stellung	Bes.	%
Linolsäure	C18:2	9c,12c	ω−6	11,1
	C18:2	9c,12t	trans	n.b.
	C18:2	9t,c12	trans	n.b.
	C18:2	9t,12t	trans	n.b.
	C18:2	9c,t11	CLA	1,2
	C18:2	10t,c12	CLA	1,0
Eicosadiensäure	C20:2	11c,14c	ω−6	0,7
Docosadiensäure	C22:2	13c,16c	ω−6	n.b.
alpha Linolensäure	C18:3	9c,11c,15c	ω−3	21,9
gamma Linolensäure	C18:3	6c,9c,12c	ω−6	n.b.
Dihomo-gamma Linolensäure	C20:3	8c,11c,14c	ω−6	0,5
Arachidonsäure	C20:4	5c,8c,11c,14c	ω−6	1,2
Docosatetraensäure	C22:4	7c,10c,13c,16c	ω−6	n.b.
Eicosapentaensäure	C20:5	5c,8c,11c,14c,17c,	ω−3	n.b.
Docosapentaensäure	C22:5	7c,10c,13c,16c,19c	ω−3	n.b.
Docosahexaensäure	C22:6	4c,7c,10c,13c,16c,19c	ω−3	n.b.

Parameter	%
Summe der gesättigten Fettsäuren	45,1
Summe der einfach ungesättigten Fettsäuren	17,2
Summe der mehrfach ungesättigten Fettsäuren	37,7
Summe der konjugierten Linolsäure-Isomere	2,2
Summe der trans-Fettsäuren	n.b.
Summe der ω−3 Fettsäuren	21,9
Summe der ω−6 Fettsäuren	13,5
Summe der ω−9 Fettsäuren	9,5

% - Angabe bezieht sich auf den Fettsäureanteil
n.b. - nicht bestimmbar

Im Auftrag

Dr. Andrea Kuhr
Leitung Rückstands- und Radioaktivitätsuntersuchungen

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Figure B.8: Lab report of the FA profile of *Azolla*.

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Prüfbericht

Oldenburg, 02.07.2019
Seite 1 von 1
Berichts-Version: 1

Kunden-Nr.:	50178422	Probeneingang:	04.06.2019
Auftrags-Nr.:	1649882	Untersuchungsbeginn:	04.06.2019
Proben-Nr.:	**19FU023252**	Untersuchungsende:	01.07.2019
Probenart:	Sonstige		
Befindlich in:	Siegeltüte, Plombe/Siegel: 84703476, 84703478, 84703490, 84703488, 84703486		
Bezeichnung:	Azolla (Algenfarn)		
Probenehmer:	durch Auftraggeber		

	Ergebnis in der Originalsubstanz	Berechnet auf 100 % Trockensubstanz
Wasser *Methode: VO (EG) 152 Anhang III, A; 2009*	**95,0 %**	
Rohasche *Methode: VO (EG) 152 Anhang III, M; 2009*	**0,7 %**	14,3 %
Rohprotein *Methode: VO (EG) 152 Anhang III, C; 2009*	**1,5 %**	29,3 %
Rohfett A+B *Methode: VO (EG) 152 Anhang III, H; 2009*	**0,3 %**	6,1 %
Stärke *Methode: VO (EG) 152 Anhang III, L; 2009*	**0,3 %**	5,8 %
Gesamtzucker ber. als Saccharose *Methode: VO (EG) 152 Anhang III, J; 2009*	**0,3 %**	6,2 %
Fettsäuremuster *Methode: ASU L 13.00-26; 2008-06 / ASU L 13.00-27/2; 2012-01*	**siehe Anlage**	

Im Auftrag

Dr. Michael Egert
Institutsleiter

Dieser Prüfbericht wurde maschinell erstellt und ist ohne Unterschrift gültig.

#2 = IfT, Oldenburg; #3 = IfL, Oldenburg; #4 = IfB, IfD, Hameln; #5 = Untersuchung erfolgte in Fremdlabor; #6 = unterliegt nicht der Akkreditierung
„<…" = Wert ist kleiner als die nebenstehende untere Grenze des Arbeitsbereiches

Figure B.9: Lab report of nutritional analysis of *Azolla*.

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http://www.lufa-nord-west.de

DAkkS Deutsche Akkreditierungsstelle D-PL-14165-01-00

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Prüfbericht

Oldenburg, 13.12.2019
Seite 1 von 1
Berichts-Version: 1

Kunden-Nr.:	50178422	Probeneingang:	05.11.2019
Auftrags-Nr.:	1850734	Untersuchungsbeginn:	05.11.2019
Proben-Nr.:	**19FU045734**	Untersuchungsende:	12.12.2019
Probenart:	Leinsaat		
Befindlich in:	Siegeltüte, Plombe/Siegel: 84703496		
Bezeichnung:	Leinsamenschrot		
Probenehmer:	durch Auftraggeber		
Probenzustand:	Probeneingang gekühlt		

	Ergebnis in der Originalsubstanz
Wasser *VO (EG) 152 Anhang III, A; 2009*	**8,6 %**
Rohasche *VO (EG) 152 Anhang III, M; 2009*	**2,8 %**
Rohprotein *VO (EG) 152 Anhang III, C; 2009*	**21,4 %**
Rohfett A+B *VO (EG) 152 Anhang III, H; 2009*	**42,7 %**
Stärke *VO (EG) 152 Anhang III, L; 2009*	**2,3 %**
Gesamtzucker ber. als Saccharose *VO (EG) 152 Anhang III, J; 2009*	**1,1 %**
Fettsäuremuster *ASU L 13.00-26; 2008-06 / ASU L 13.00-27/2; 2012-01*	**siehe Anlage**

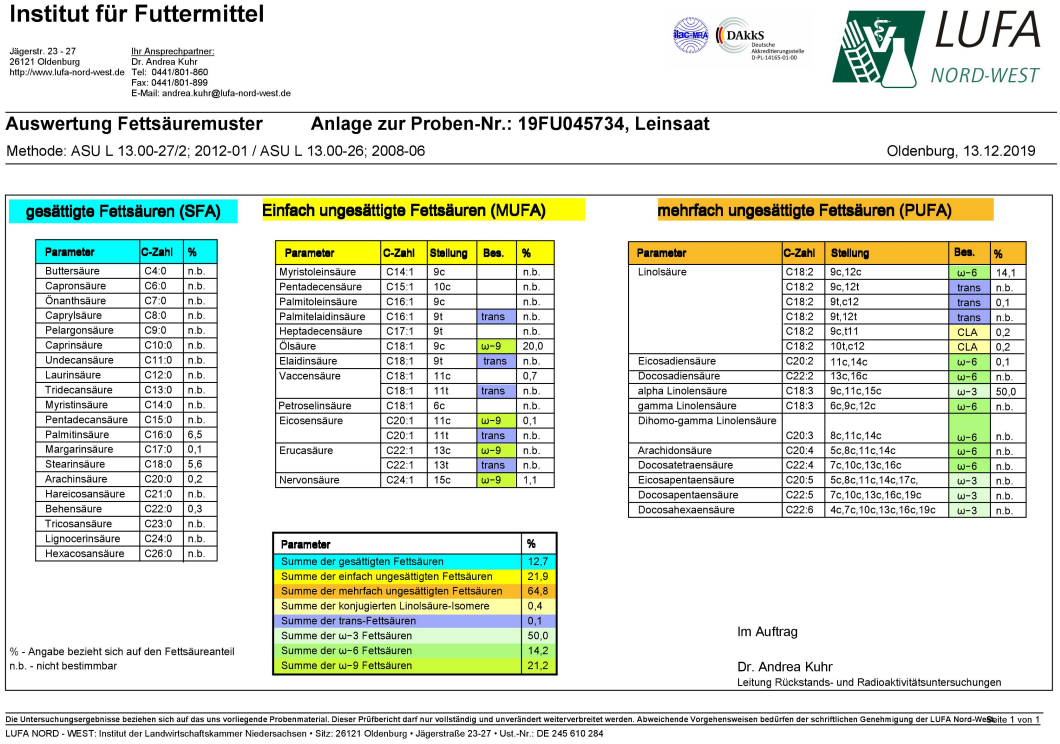
Im Auftrag

Dr. Michael Egert
Institutsleiter

Dieser Prüfbericht wurde maschinell erstellt und ist ohne Unterschrift gültig.

#2 = IfT, Oldenburg; #3 = IfL, Oldenburg; #4 = IfB, IfD, Hameln; #5 = Untersuchung erfolgte in Fremdlabor; #6 = unterliegt nicht der Akkreditierung
„<…" = Wert ist kleiner als die nebenstehende untere Grenze des Arbeitsbereiches

Figure B.10: Lab report of the nutritional analysis of flax seed.



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LUFA NORD-WEST

Auswertung Fettsäuremuster **Anlage zur Proben-Nr.: 19FU045734, Leinsaat**

Methode: ASU L 13.00-27/2; 2012-01 / ASU L 13.00-26; 2008-06 Oldenburg, 13.12.2019

gesättigte Fettsäuren (SFA)

Parameter	C-Zahl	%
Buttersäure	C4:0	n.b.
Capronsäure	C6:0	n.b.
Önanthsäure	C7:0	n.b.
Caprylsäure	C8:0	n.b.
Pelargonsäure	C9:0	n.b.
Caprinsäure	C10:0	n.b.
Undecansäure	C11:0	n.b.
Laurinsäure	C12:0	n.b.
Tridecansäure	C13:0	n.b.
Myristinsäure	C14:0	n.b.
Pentadecansäure	C15:0	n.b.
Palmitinsäure	C16:0	6,5
Margarinsäure	C17:0	0,1
Stearinsäure	C18:0	5,6
Arachinsäure	C20:0	0,2
Hareicosansäure	C21:0	n.b.
Behensäure	C22:0	0,3
Tricosansäure	C23:0	n.b.
Lignocerinsäure	C24:0	n.b.
Hexacosansäure	C26:0	n.b.

Einfach ungesättigte Fettsäuren (MUFA)

Parameter	C-Zahl	Stellung	Bes.	%
Myristoleinsäure	C14:1	9c		n.b.
Pentadecensäure	C15:1	10c		n.b.
Palmitoleinsäure	C16:1	9c		n.b.
Palmitelaidinsäure	C16:1	9t	trans	n.b.
Heptadecensäure	C17:1	9t		n.b.
Ölsäure	C18:1	9c	ω−9	20,0
Elaidinsäure	C18:1	9t	trans	n.b.
Vaccensäure	C18:1	11c		0,7
	C18:1	11t	trans	n.b.
Petroselinsäure	C18:1	6c		n.b.
Eicosensäure	C20:1	11c	ω−9	0,1
	C20:1	11t	trans	n.b.
Erucasäure	C22:1	13c	ω−9	n.b.
	C22:1	13t	trans	n.b.
Nervonsäure	C24:1	15c	ω−9	1,1

mehrfach ungesättigte Fettsäuren (PUFA)

Parameter	C-Zahl	Stellung	Bes.	%
Linolsäure	C18:2	9c,12c	ω−6	14,1
	C18:2	9c,12t	trans	n.b.
	C18:2	9t,c12	trans	0,1
	C18:2	9t,12t	trans	n.b.
	C18:2	9c,t11	CLA	0,2
	C18:2	10t,c12	CLA	0,2
Eicosadiensäure	C20:2	11c,14c	ω−6	0,1
Docosadiensäure	C22:2	13c,16c	ω−6	n.b.
alpha Linolensäure	C18:3	9c,11c,15c	ω−3	50,0
gamma Linolensäure	C18:3	6c,9c,12c	ω−6	n.b.
Dihomo-gamma Linolensäure	C20:3	8c,11c,14c	ω−6	n.b.
Arachidonsäure	C20:4	5c,8c,11c,14c	ω−6	n.b.
Docosatetraensäure	C22:4	7c,10c,13c,16c	ω−6	n.b.
Eicosapentaensäure	C20:5	5c,8c,11c,14c,17c,	ω−3	n.b.
Docosapentaensäure	C22:5	7c,10c,13c,16c,19c	ω−3	n.b.
Docosahexaensäure	C22:6	4c,7c,10c,13c,16c,19c	ω−3	n.b.

Parameter	%
Summe der gesättigten Fettsäuren	12,7
Summe der einfach ungesättigten Fettsäuren	21,9
Summe der mehrfach ungesättigten Fettsäuren	64,8
Summe der konjugierten Linolsäure-Isomere	0,4
Summe der trans-Fettsäuren	0,1
Summe der ω−3 Fettsäuren	50,0
Summe der ω−6 Fettsäuren	14,2
Summe der ω−9 Fettsäuren	21,2

% - Angabe bezieht sich auf den Fettsäureanteil
n.b. - nicht bestimmbar

Im Auftrag

Dr. Andrea Kuhr
Leitung Rückstands- und Radioaktivitätsuntersuchungen

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Figure B.11: Lab report of the FA profile of flax seed.

Appendix C

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Prüfbericht

Oldenburg, 10.05.2019
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Berichts-Version: 1

Kunden-Nr.:	50178422	Probeneingang:	03.05.2019
Auftrags-Nr.:	1613992	Untersuchungsbeginn:	03.05.2019
Proben-Nr.:	**19FU018848**	Untersuchungsende:	10.05.2019
Probenart:	Eier & Eiprodukte		
Befindlich in:	Pappkarton		
Bezeichnung:	Kontrollgruppe - Ei-Inhalt		
Probenehmer:	durch Auftraggeber		

	Ergebnis in der Originalsubstanz
Rohfett A+B *Methode: VO (EG) 152 Anhang III, H; 2009*	**10,1 %**
Vitamin A *Methode: LUFA Nord-West 1/3-029; 2017-11*	**9940 IE/kg**
Vitamin E, berechnet als alpha-Tocopherolacetat *Methode: LUFA Nord-West 1/3-029; 2017-11*	**44 mg/kg**
Fettsäuremuster *Methode: ASU L 13.00-26; 2008-06 / ASU L 13.00-27/2; 2012-01*	**siehe Anlage**
Mikrobiologische Untersuchungsergebnisse	
Salmonella spp. *Methode: DIN EN ISO 6579-1; 2017-07*	**nicht nachgewiesen in 25g**

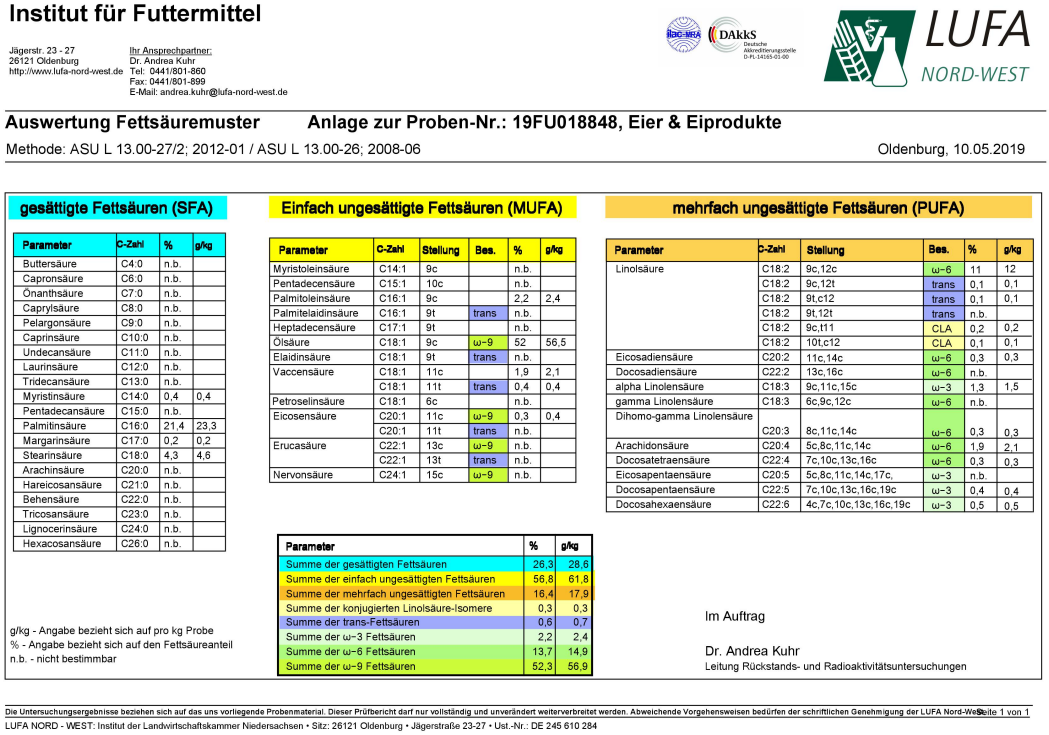
Im Auftrag

Dr. Michael Egert
Institutsleiter

Dieser Prüfbericht wurde maschinell erstellt und ist ohne Unterschrift gültig.

#2 = IfT, Oldenburg; #3 = IfL, Oldenburg; #4 = IfB, IfD, Hameln; #5 = Untersuchung erfolgte in Fremdlabor; #6 = unterliegt nicht der Akkreditierung
„<…" = Wert ist kleiner als die nebenstehende untere Grenze des Arbeitsbereiches

Figure C.1: Lab report of the nutritional analysis of the duck eggs of the control group (Experiment 1).



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LUFA NORD-WEST

Auswertung Fettsäuremuster **Anlage zur Proben-Nr.: 19FU018848, Eier & Eiprodukte**

Methode: ASU L 13.00-27/2; 2012-01 / ASU L 13.00-26; 2008-06 Oldenburg, 10.05.2019

gesättigte Fettsäuren (SFA)

Parameter	C-Zahl	%	g/kg
Buttersäure	C4:0	n.b.	
Capronsäure	C6:0	n.b.	
Önanthsäure	C7:0	n.b.	
Caprylsäure	C8:0	n.b.	
Pelargonsäure	C9:0	n.b.	
Caprinsäure	C10:0	n.b.	
Undecansäure	C11:0	n.b.	
Laurinsäure	C12:0	n.b.	
Tridecansäure	C13:0	n.b.	
Myristinsäure	C14:0	0,4	0,4
Pentadecansäure	C15:0	n.b.	
Palmitinsäure	C16:0	21,4	23,3
Margarinsäure	C17:0	0,2	0,2
Stearinsäure	C18:0	4,3	4,6
Arachinsäure	C20:0	n.b.	
Hareicosansäure	C21:0	n.b.	
Behensäure	C22:0	n.b.	
Tricosansäure	C23:0	n.b.	
Lignocerinsäure	C24:0	n.b.	
Hexacosansäure	C26:0	n.b.	

Einfach ungesättigte Fettsäuren (MUFA)

Parameter	C-Zahl	Stellung	Bes.	%	g/kg
Myristoleinsäure	C14:1	9c		n.b.	
Pentadecensäure	C15:1	10c		n.b.	
Palmitoleinsäure	C16:1	9c		2,2	2,4
Palmitelaidinsäure	C16:1	9t	trans	n.b.	
Heptadecensäure	C17:1	9t		n.b.	
Ölsäure	C18:1	9c	ω-9	52	56,5
Elaidinsäure	C18:1	9t	trans	n.b.	
Vaccensäure	C18:1	11c		1,9	2,1
	C18:1	11t	trans	0,4	0,4
Petroselinsäure	C18:1	6c		n.b.	
Eicosensäure	C20:1	11c	ω-9	0,3	0,4
	C20:1	11t	trans	n.b.	
Erucasäure	C22:1	13c	ω-9	n.b.	
	C22:1	13t	trans	n.b.	
Nervonsäure	C24:1	15c	ω-9	n.b.	

mehrfach ungesättigte Fettsäuren (PUFA)

Parameter	C-Zahl	Stellung	Bes.	%	g/kg
Linolsäure	C18:2	9c,12c	ω-6	11	12
	C18:2	9c,12t	trans	0,1	0,1
	C18:2	9t,c12	trans	0,1	0,1
	C18:2	9t,12t	trans	n.b.	
	C18:2	9c,t11	CLA	0,2	0,2
	C18:2	10t,c12	CLA	0,1	0,1
Eicosadiensäure	C20:2	11c,14c	ω-6	0,3	0,3
Docosadiensäure	C22:2	13c,16c	ω-6	n.b.	
alpha Linolensäure	C18:3	9c,11c,15c	ω-3	1,3	1,5
gamma Linolensäure	C18:3	6c,9c,12c	ω-6	n.b.	
Dihomo-gamma Linolensäure	C20:3	8c,11c,14c	ω-6	0,3	0,3
Arachidonsäure	C20:4	5c,8c,11c,14c	ω-6	1,9	2,1
Docosatetraensäure	C22:4	7c,10c,13c,16c	ω-6	0,3	0,3
Eicosapentaensäure	C20:5	5c,8c,11c,14c,17c,	ω-3	n.b.	
Docosapentaensäure	C22:5	7c,10c,13c,16c,19c	ω-3	0,4	0,4
Docosahexaensäure	C22:6	4c,7c,10c,13c,16c,19c	ω-3	0,5	0,5

Parameter	%	g/kg
Summe der gesättigten Fettsäuren	26,3	28,6
Summe der einfach ungesättigten Fettsäuren	56,8	61,8
Summe der mehrfach ungesättigten Fettsäuren	16,4	17,9
Summe der konjugierten Linolsäure-Isomere	0,3	0,3
Summe der trans-Fettsäuren	0,6	0,7
Summe der ω-3 Fettsäuren	2,2	2,4
Summe der ω-6 Fettsäuren	13,7	14,9
Summe der ω-9 Fettsäuren	52,3	56,9

g/kg - Angabe bezieht sich auf pro kg Probe
% - Angabe bezieht sich auf den Fettsäureanteil
n.b. - nicht bestimmbar

Im Auftrag

Dr. Andrea Kuhr
Leitung Rückstands- und Radioaktivitätsuntersuchungen

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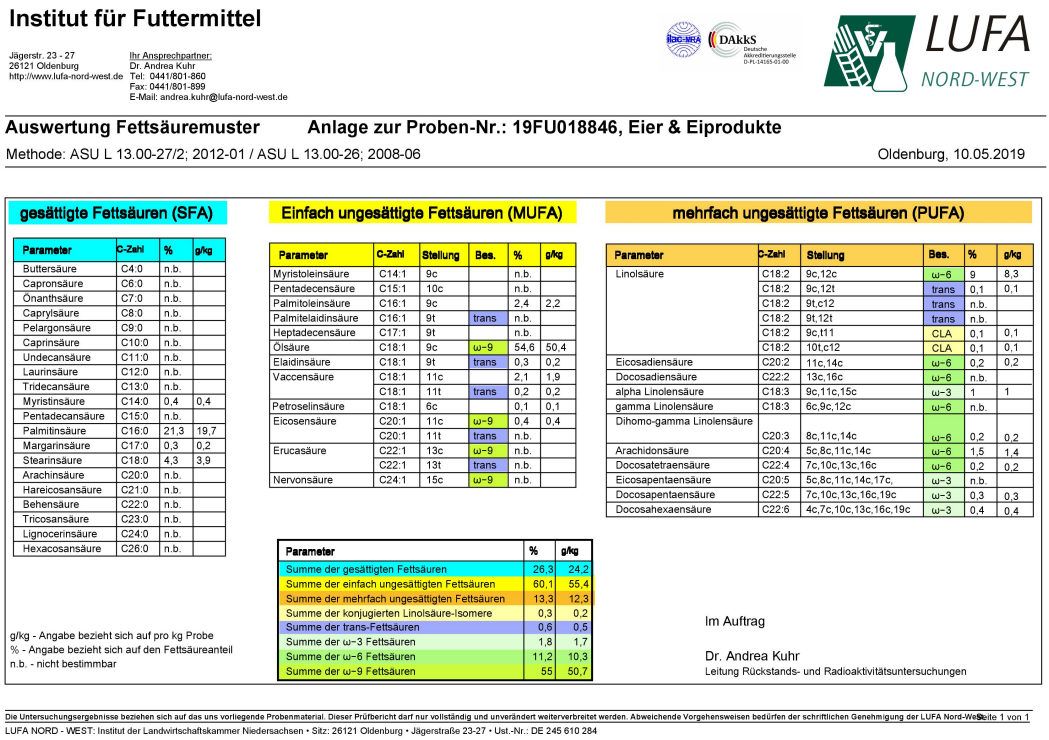
Figure C.2: Lab report of the FA profile of the duck eggs of the control group (Experiment 1).



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LUFA NORD-WEST

Auswertung Fettsäuremuster **Anlage zur Proben-Nr.: 19FU018846, Eier & Eiprodukte**

Methode: ASU L 13.00-27/2; 2012-01 / ASU L 13.00-26; 2008-06 Oldenburg, 10.05.2019

gesättigte Fettsäuren (SFA)

Parameter	C-Zahl	%	g/kg
Buttersäure	C4:0	n.b.	
Capronsäure	C6:0	n.b.	
Önanthsäure	C7:0	n.b.	
Caprylsäure	C8:0	n.b.	
Pelargonsäure	C9:0	n.b.	
Caprinsäure	C10:0	n.b.	
Undecansäure	C11:0	n.b.	
Laurinsäure	C12:0	n.b.	
Tridecansäure	C13:0	n.b.	
Myristinsäure	C14:0	0,4	0,4
Pentadecansäure	C15:0	n.b.	
Palmitinsäure	C16:0	21,3	19,7
Margarinsäure	C17:0	0,3	0,2
Stearinsäure	C18:0	4,3	3,9
Arachinsäure	C20:0	n.b.	
Hareicosansäure	C21:0	n.b.	
Behensäure	C22:0	n.b.	
Tricosansäure	C23:0	n.b.	
Lignocerinsäure	C24:0	n.b.	
Hexacosansäure	C26:0	n.b.	

Einfach ungesättigte Fettsäuren (MUFA)

Parameter	C-Zahl	Stellung	Bes.	%	g/kg
Myristoleinsäure	C14:1	9c		n.b.	
Pentadecensäure	C15:1	10c		n.b.	
Palmitoleinsäure	C16:1	9c		2,4	2,2
Palmitelaidinsäure	C16:1	9t	trans	n.b.	
Heptadecensäure	C17:1	9t		n.b.	
Ölsäure	C18:1	9c	ω-9	54,6	50,4
Elaidinsäure	C18:1	9t	trans	0,3	0,2
Vaccensäure	C18:1	11c		2,1	1,9
	C18:1	11t	trans	0,2	0,2
Petroselinsäure	C18:1	6c		0,1	0,1
Eicosensäure	C20:1	11c	ω-9	0,4	0,4
	C20:1	11t	trans	n.b.	
Erucasäure	C22:1	13c	ω-9	n.b.	
	C22:1	13t	trans	n.b.	
Nervonsäure	C24:1	15c	ω-9	n.b.	

mehrfach ungesättigte Fettsäuren (PUFA)

Parameter	C-Zahl	Stellung	Bes.	%	g/kg
Linolsäure	C18:2	9c,12c	ω-6	9	8,3
	C18:2	9c,12t	trans	0,1	0,1
	C18:2	9t,c12	trans	n.b.	
	C18:2	9t,12t	trans	n.b.	
	C18:2	9c,t11	CLA	0,1	0,1
	C18:2	10t,c12	CLA	0,1	0,1
Eicosadiensäure	C20:2	11c,14c	ω-6	0,2	0,2
Docosadiensäure	C22:2	13c,16c	ω-6	n.b.	
alpha Linolensäure	C18:3	9c,11c,15c	ω-3	1	1
gamma Linolensäure	C18:3	6c,9c,12c	ω-6	n.b.	
Dihomo-gamma Linolensäure	C20:3	8c,11c,14c	ω-6	0,2	0,2
Arachidonsäure	C20:4	5c,8c,11c,14c	ω-6	1,5	1,4
Docosatetraensäure	C22:4	7c,10c,13c,16c	ω-6	0,2	0,2
Eicosapentaensäure	C20:5	5c,8c,11c,14c,17c,	ω-3	n.b.	
Docosapentaensäure	C22:5	7c,10c,13c,16c,19c	ω-3	0,3	0,3
Docosahexaensäure	C22:6	4c,7c,10c,13c,16c,19c	ω-3	0,4	0,4

Parameter	%	g/kg
Summe der gesättigten Fettsäuren	26,3	24,2
Summe der einfach ungesättigten Fettsäuren	60,1	55,4
Summe der mehrfach ungesättigten Fettsäuren	13,3	12,3
Summe der konjugierten Linolsäure-Isomere	0,3	0,2
Summe der trans-Fettsäuren	0,6	0,5
Summe der ω-3 Fettsäuren	1,8	1,7
Summe der ω-6 Fettsäuren	11,2	10,3
Summe der ω-9 Fettsäuren	55	50,7

g/kg - Angabe bezieht sich auf pro kg Probe
% - Angabe bezieht sich auf den Fettsäureanteil
n.b. - nicht bestimmbar

Im Auftrag

Dr. Andrea Kuhr
Leitung Rückstands- und Radioaktivitätsuntersuchungen

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Figure C.3: Lab report of the FA profile of the duck eggs of the experimental group (Experiment 1).

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Prüfbericht

Oldenburg, 10.05.2019
Seite 1 von 1
Berichts-Version: 1

Kunden-Nr.:	50178422	Probeneingang:	03.05.2019
Auftrags-Nr.:	1613992	Untersuchungsbeginn:	03.05.2019
Proben-Nr.:	**19FU018846**	Untersuchungsende:	10.05.2019
Probenart:	Eier & Eiprodukte		
Befindlich in:	Pappkarton		
Bezeichnung:	Versuchsgruppe - Ei-Inhalt		
Probenehmer:	durch Auftraggeber		

	Ergebnis in der Originalsubstanz
Rohfett A+B *Methode: VO (EG) 152 Anhang III, H; 2009*	**10,9 %**
Vitamin A *Methode: LUFA Nord-West 1/3-029; 2017-11*	**8580 IE/kg**
Vitamin E, berechnet als alpha-Tocopherolacetat *Methode: LUFA Nord-West 1/3-029; 2017-11*	**46 mg/kg**
Fettsäuremuster *Methode: ASU L 13.00-26; 2008-06 / ASU L 13.00-27/2; 2012-01*	**siehe Anlage**
Mikrobiologische Untersuchungsergebnisse	
Salmonella spp. *Methode: DIN EN ISO 6579-1; 2017-07*	**nicht nachgewiesen in 25g**

Im Auftrag

Dr. Michael Egert
Institutsleiter

Dieser Prüfbericht wurde maschinell erstellt und ist ohne Unterschrift gültig.

#2 = IfT, Oldenburg; #3 = IfL, Oldenburg; #4 = IfB, IfD, Hameln; #5 = Untersuchung erfolgte in Fremdlabor; #6 = unterliegt nicht der Akkreditierung
„<…" = Wert ist kleiner als die nebenstehende untere Grenze des Arbeitsbereiches

Die Untersuchungsergebnisse beziehen sich auf das uns vorliegende Probenmaterial. Dieser Prüfbericht darf nur vollständig und unverändert weiterverbreitet werden. Abweichende Vorgehensweisen bedürfen der schriftlichen Genehmigung der LUFA Nord-West. Die Akkreditierung gilt für den in der Urkundenanlage D-PL-14165-01-00 festgelegten Umfang.

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Figure C.4: Lab report of the nutritional analysis of the duck eggs of the experimental group (Experiment 1).

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Prüfbericht

Oldenburg, 14.05.2019
Seite 1 von 1
Berichts-Version: 1

Kunden-Nr.:	50178422	Probeneingang:	03.05.2019
Auftrags-Nr.:	1613992	Untersuchungsbeginn:	03.05.2019
Proben-Nr.:	**19FU018849**	Untersuchungsende:	09.05.2019
Probenart:	Eierschalen		
Befindlich in:	Pappkarton		
Bezeichnung:	Kontrollgruppe - Eierschalen		
Probenehmer:	durch Auftraggeber		

	Ergebnis in der Originalsubstanz
Mikrobiologische Untersuchungsergebnisse	
Salmonella spp. *Methode: DIN EN ISO 6579-1; 2017-07*	**nicht nachgewiesen in 25g**

Im Auftrag

Dr. Hartwig Wellmann
Laborbereichsleiter

Dieser Prüfbericht wurde maschinell erstellt und ist ohne Unterschrift gültig.

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„<…" = Wert ist kleiner als die nebenstehende untere Grenze des Arbeitsbereiches

Die Untersuchungsergebnisse beziehen sich auf das uns vorliegende Probenmaterial. Dieser Prüfbericht darf nur vollständig und unverändert weiterverbreitet werden. Abweichende Vorgehensweisen bedürfen der schriftlichen Genehmigung der LUFA Nord-West. Die Akkreditierung gilt für den in der Urkundenanlage D-PL-14165-01-00 festgelegten Umfang.

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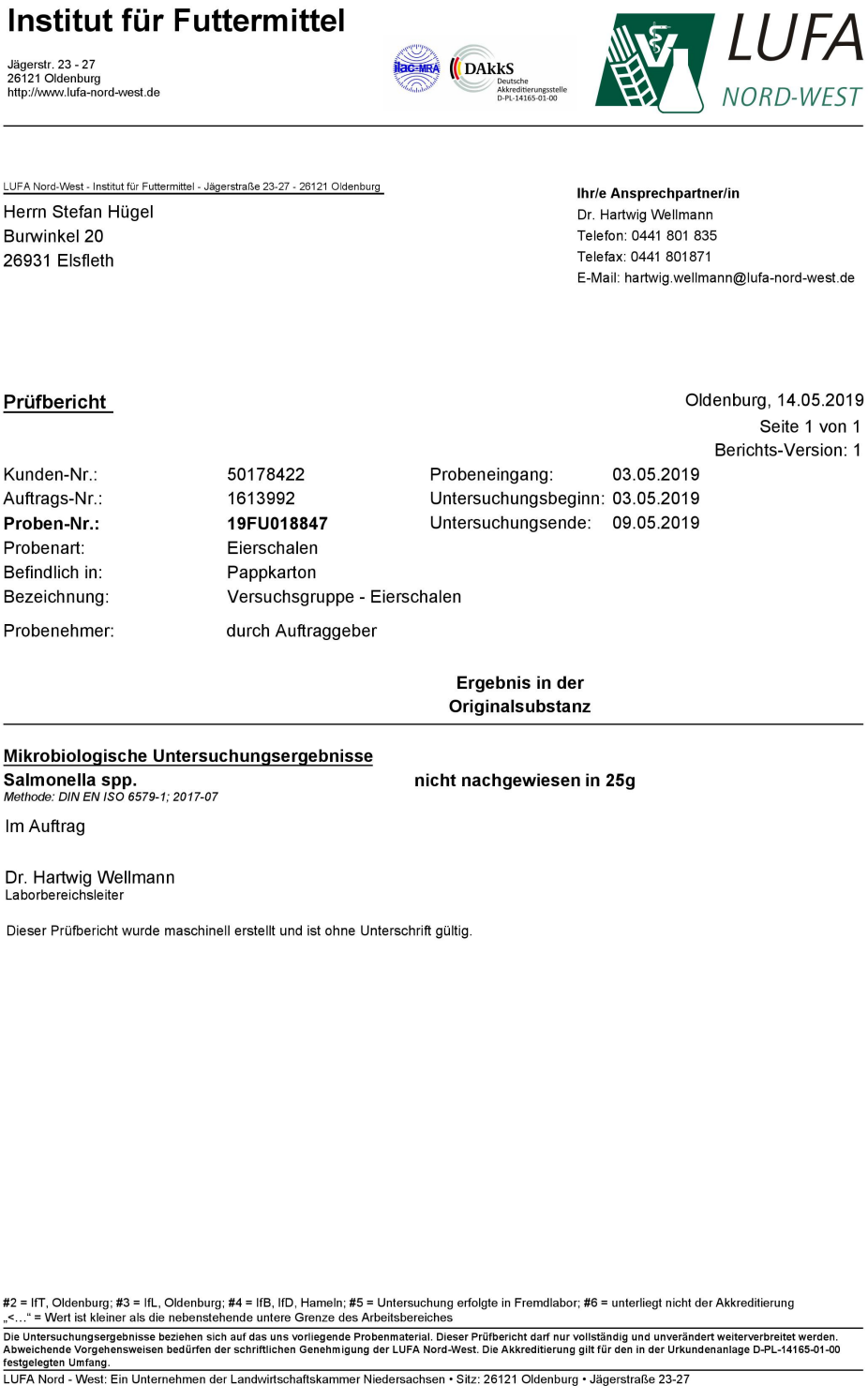
Figure C.5: Lab report of the Salmonella analysis of the duck eggs of the control group (Experiment 1).



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Prüfbericht

Oldenburg, 14.05.2019
Seite 1 von 1
Berichts-Version: 1

Kunden-Nr.:	50178422	Probeneingang:	03.05.2019
Auftrags-Nr.:	1613992	Untersuchungsbeginn:	03.05.2019
Proben-Nr.:	**19FU018847**	Untersuchungsende:	09.05.2019
Probenart:	Eierschalen		
Befindlich in:	Pappkarton		
Bezeichnung:	Versuchsgruppe - Eierschalen		
Probenehmer:	durch Auftraggeber		

	Ergebnis in der Originalsubstanz
Mikrobiologische Untersuchungsergebnisse	
Salmonella spp. *Methode: DIN EN ISO 6579-1; 2017-07*	**nicht nachgewiesen in 25g**

Im Auftrag

Dr. Hartwig Wellmann
Laborbereichsleiter

Dieser Prüfbericht wurde maschinell erstellt und ist ohne Unterschrift gültig.

#2 = IfT, Oldenburg; #3 = IfL, Oldenburg; #4 = IfB, IfD, Hameln; #5 = Untersuchung erfolgte in Fremdlabor; #6 = unterliegt nicht der Akkreditierung
„<…" = Wert ist kleiner als die nebenstehende untere Grenze des Arbeitsbereiches

Die Untersuchungsergebnisse beziehen sich auf das uns vorliegende Probenmaterial. Dieser Prüfbericht darf nur vollständig und unverändert weiterverbreitet werden. Abweichende Vorgehensweisen bedürfen der schriftlichen Genehmigung der LUFA Nord-West. Die Akkreditierung gilt für den in der Urkundenanlage D-PL-14165-01-00 festgelegten Umfang.

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Figure C.6: Lab report of the Salmonella analysis of the duck eggs of the experimental group (Experiment 1).

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Prüfbericht

Oldenburg, 29.08.2019
Seite 1 von 1
Berichts-Version: 1

Kunden-Nr.:	50178422	Probeneingang:	19.08.2019
Auftrags-Nr.:	1745198	Untersuchungsbeginn:	19.08.2019
Proben-Nr.:	**19FU033990**	Untersuchungsende:	29.08.2019
Probenart:	Eier & Eiprodukte		
Befindlich in:	Pappkarton		
Bezeichnung:	K 29./30.7.19		
Probenehmer:	durch Auftraggeber		

	Ergebnis in der Originalsubstanz
Rohfett A+B *VO (EG) 152 Anhang III, H; 2009*	**14,4 %**
Vitamin A *LUFA Nord-West 1/3-029; 2017-11*	**10700 IE/kg**
Vitamin E, berechnet als alpha-Tocopherolacetat *LUFA Nord-West 1/3-029; 2017-11*	**49 mg/kg**
Fettsäuremuster *ASU L 13.00-26; 2008-06 / ASU L 13.00-27/2; 2012-01*	**siehe Anlage**

Im Auftrag

Dr. Hartwig Wellmann
Laborbereichsleiter

Dieser Prüfbericht wurde maschinell erstellt und ist ohne Unterschrift gültig.

#2 = IfT, Oldenburg; #3 = IfL, Oldenburg; #4 = IfB, IfD, Hameln; #5 = Untersuchung erfolgte in Fremdlabor; #6 = unterliegt nicht der Akkreditierung
„<…" = Wert ist kleiner als die nebenstehende untere Grenze des Arbeitsbereiches

Die Untersuchungsergebnisse beziehen sich auf das uns vorliegende Probenmaterial. Dieser Prüfbericht darf nur vollständig und unverändert weiterverbreitet werden. Abweichende Vorgehensweisen bedürfen der schriftlichen Genehmigung der LUFA Nord-West. Die Akkreditierung gilt für den in der Urkundenanlage D-PL-14165-01-00 festgelegten Umfang.

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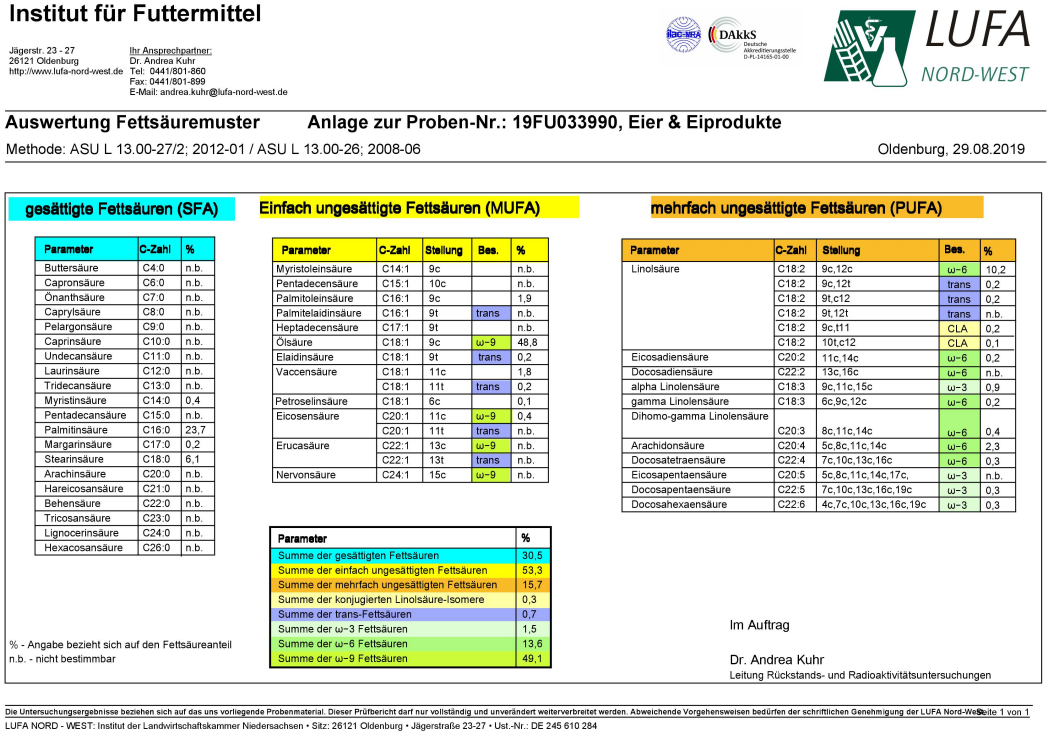
Figure C.7: Lab report of the nutritional analysis of the duck eggs of the control group (Experiment 2).



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LUFA NORD-WEST

Auswertung Fettsäuremuster **Anlage zur Proben-Nr.: 19FU033990, Eier & Eiprodukte**

Methode: ASU L 13.00-27/2; 2012-01 / ASU L 13.00-26; 2008-06 Oldenburg, 29.08.2019

gesättigte Fettsäuren (SFA)

Parameter	C-Zahl	%
Buttersäure	C4:0	n.b.
Capronsäure	C6:0	n.b.
Önanthsäure	C7:0	n.b.
Caprylsäure	C8:0	n.b.
Pelargonsäure	C9:0	n.b.
Caprinsäure	C10:0	n.b.
Undecansäure	C11:0	n.b.
Laurinsäure	C12:0	n.b.
Tridecansäure	C13:0	n.b.
Myristinsäure	C14:0	0,4
Pentadecansäure	C15:0	n.b.
Palmitinsäure	C16:0	23,7
Margarinsäure	C17:0	0,2
Stearinsäure	C18:0	6,1
Arachinsäure	C20:0	n.b.
Hareicosansäure	C21:0	n.b.
Behensäure	C22:0	n.b.
Tricosansäure	C23:0	n.b.
Lignocerinsäure	C24:0	n.b.
Hexacosansäure	C26:0	n.b.

Einfach ungesättigte Fettsäuren (MUFA)

Parameter	C-Zahl	Stellung	Bes.	%
Myristoleinsäure	C14:1	9c		n.b.
Pentadecensäure	C15:1	10c		n.b.
Palmitoleinsäure	C16:1	9c		1,9
Palmitelaidinsäure	C16:1	9t	trans	n.b.
Heptadecensäure	C17:1	9t		n.b.
Ölsäure	C18:1	9c	ω-9	48,8
Elaidinsäure	C18:1	9t	trans	0,2
Vaccensäure	C18:1	11c		1,8
	C18:1	11t	trans	0,2
Petroselinsäure	C18:1	6c		0,1
Eicosensäure	C20:1	11c	ω-9	0,4
	C20:1	11t	trans	n.b.
Erucasäure	C22:1	13c	ω-9	n.b.
	C22:1	13t	trans	n.b.
Nervonsäure	C24:1	15c	ω-9	n.b.

mehrfach ungesättigte Fettsäuren (PUFA)

Parameter	C-Zahl	Stellung	Bes.	%
Linolsäure	C18:2	9c,12c	ω-6	10,2
	C18:2	9c,12t	trans	0,2
	C18:2	9t,c12	trans	0,2
	C18:2	9t,12t	trans	n.b.
	C18:2	9c,t11	CLA	0,2
	C18:2	10t,c12	CLA	0,1
Eicosadiensäure	C20:2	11c,14c	ω-6	0,2
Docosadiensäure	C22:2	13c,16c	ω-6	n.b.
alpha Linolensäure	C18:3	9c,11c,15c	ω-3	0,9
gamma Linolensäure	C18:3	6c,9c,12c	ω-6	0,2
Dihomo-gamma Linolensäure	C20:3	8c,11c,14c	ω-6	0,4
Arachidonsäure	C20:4	5c,8c,11c,14c	ω-6	2,3
Docosatetraensäure	C22:4	7c,10c,13c,16c	ω-6	0,3
Eicosapentaensäure	C20:5	5c,8c,11c,14c,17c,	ω-3	n.b.
Docosapentaensäure	C22:5	7c,10c,13c,16c,19c	ω-3	0,3
Docosahexaensäure	C22:6	4c,7c,10c,13c,16c,19c	ω-3	0,3

Parameter	%
Summe der gesättigten Fettsäuren	30,5
Summe der einfach ungesättigten Fettsäuren	53,3
Summe der mehrfach ungesättigten Fettsäuren	15,7
Summe der konjugierten Linolsäure-Isomere	0,3
Summe der trans-Fettsäuren	0,7
Summe der ω-3 Fettsäuren	1,5
Summe der ω-6 Fettsäuren	13,6
Summe der ω-9 Fettsäuren	49,1

% - Angabe bezieht sich auf den Fettsäureanteil
n.b. - nicht bestimmbar

Im Auftrag

Dr. Andrea Kuhr
Leitung Rückstands- und Radioaktivitätsuntersuchungen

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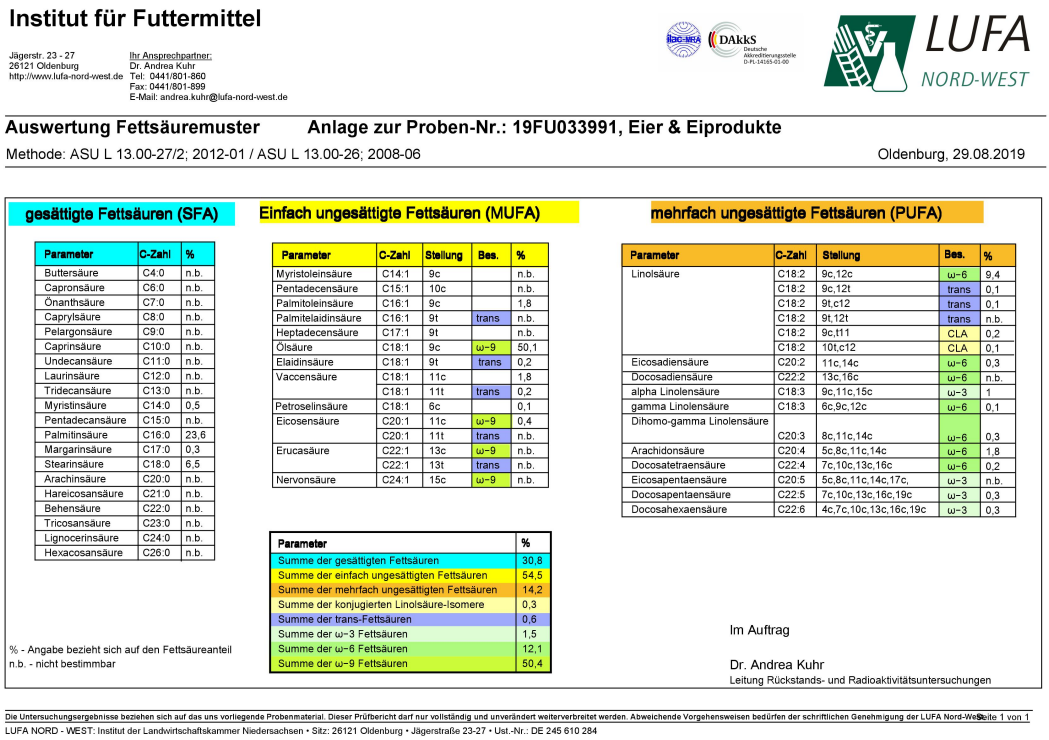
Figure C.8: Lab report of the FA profile of the duck eggs of the control group (Experiment 2).



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LUFA NORD-WEST

Auswertung Fettsäuremuster **Anlage zur Proben-Nr.: 19FU033991, Eier & Eiprodukte**

Methode: ASU L 13.00-27/2; 2012-01 / ASU L 13.00-26; 2008-06 Oldenburg, 29.08.2019

gesättigte Fettsäuren (SFA)

Parameter	C-Zahl	%
Buttersäure	C4:0	n.b.
Capronsäure	C6:0	n.b.
Önanthsäure	C7:0	n.b.
Caprylsäure	C8:0	n.b.
Pelargonsäure	C9:0	n.b.
Caprinsäure	C10:0	n.b.
Undecansäure	C11:0	n.b.
Laurinsäure	C12:0	n.b.
Tridecansäure	C13:0	n.b.
Myristinsäure	C14:0	0,5
Pentadecansäure	C15:0	n.b.
Palmitinsäure	C16:0	23,6
Margarinsäure	C17:0	0,3
Stearinsäure	C18:0	6,5
Arachinsäure	C20:0	n.b.
Hareicosansäure	C21:0	n.b.
Behensäure	C22:0	n.b.
Tricosansäure	C23:0	n.b.
Lignocerinsäure	C24:0	n.b.
Hexacosansäure	C26:0	n.b.

Einfach ungesättigte Fettsäuren (MUFA)

Parameter	C-Zahl	Stellung	Bes.	%
Myristoleinsäure	C14:1	9c		n.b.
Pentadecensäure	C15:1	10c		n.b.
Palmitoleinsäure	C16:1	9c		1,8
Palmitelaidinsäure	C16:1	9t	trans	n.b.
Heptadecensäure	C17:1	9t		n.b.
Ölsäure	C18:1	9c	ω-9	50,1
Elaidinsäure	C18:1	9t	trans	0,2
Vaccensäure	C18:1	11c		1,8
	C18:1	11t	trans	0,2
Petroselinsäure	C18:1	6c		0,1
Eicosensäure	C20:1	11c	ω-9	0,4
	C20:1	11t	trans	n.b.
Erucasäure	C22:1	13c	ω-9	n.b.
	C22:1	13t	trans	n.b.
Nervonsäure	C24:1	15c	ω-9	n.b.

mehrfach ungesättigte Fettsäuren (PUFA)

Parameter	C-Zahl	Stellung	Bes.	%
Linolsäure	C18:2	9c,12c	ω-6	9,4
	C18:2	9c,12t	trans	0,1
	C18:2	9t,c12	trans	0,1
	C18:2	9t,12t	trans	n.b.
	C18:2	9c,t11	CLA	0,2
	C18:2	10t,c12	CLA	0,1
Eicosadiensäure	C20:2	11c,14c	ω-6	0,3
Docosadiensäure	C22:2	13c,16c	ω-6	n.b.
alpha Linolensäure	C18:3	9c,11c,15c	ω-3	1
gamma Linolensäure	C18:3	6c,9c,12c	ω-6	0,1
Dihomo-gamma Linolensäure	C20:3	8c,11c,14c	ω-6	0,3
Arachidonsäure	C20:4	5c,8c,11c,14c	ω-6	1,8
Docosatetraensäure	C22:4	7c,10c,13c,16c	ω-6	0,2
Eicosapentaensäure	C20:5	5c,8c,11c,14c,17c,	ω-3	n.b.
Docosapentaensäure	C22:5	7c,10c,13c,16c,19c	ω-3	0,3
Docosahexaensäure	C22:6	4c,7c,10c,13c,16c,19c	ω-3	0,3

Parameter	%
Summe der gesättigten Fettsäuren	30,8
Summe der einfach ungesättigten Fettsäuren	54,5
Summe der mehrfach ungesättigten Fettsäuren	14,2
Summe der konjugierten Linolsäure-Isomere	0,3
Summe der trans-Fettsäuren	0,6
Summe der ω-3 Fettsäuren	1,5
Summe der ω-6 Fettsäuren	12,1
Summe der ω-9 Fettsäuren	50,4

% - Angabe bezieht sich auf den Fettsäureanteil
n.b. - nicht bestimmbar

Im Auftrag

Dr. Andrea Kuhr
Leitung Rückstands- und Radioaktivitätsuntersuchungen

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Figure C.9: Lab report of the FA profile of the duck eggs of the experimental group (Experiment 2).

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Prüfbericht

Oldenburg, 29.08.2019
Seite 1 von 1
Berichts-Version: 1

Kunden-Nr.:	50178422	Probeneingang:	19.08.2019
Auftrags-Nr.:	1745198	Untersuchungsbeginn:	19.08.2019
Proben-Nr.:	**19FU033991**	Untersuchungsende:	29.08.2019
Probenart:	Eier & Eiprodukte		
Befindlich in:	Pappkarton		
Bezeichnung:	V 29./30.7.19		
Probenehmer:	durch Auftraggeber		

	Ergebnis in der Originalsubstanz
Rohfett A+B *VO (EG) 152 Anhang III, H; 2009*	**15,6 %**
Vitamin A *LUFA Nord-West 1/3-029; 2017-11*	**11300 IE/kg**
Vitamin E, berechnet als alpha-Tocopherolacetat *LUFA Nord-West 1/3-029; 2017-11*	**39 mg/kg**
Fettsäuremuster *ASU L 13.00-26; 2008-06 / ASU L 13.00-27/2; 2012-01*	**siehe Anlage**

Im Auftrag

Dr. Hartwig Wellmann
Laborbereichsleiter

Dieser Prüfbericht wurde maschinell erstellt und ist ohne Unterschrift gültig.

#2 = IfT, Oldenburg; #3 = IfL, Oldenburg; #4 = IfB, IfD, Hameln; #5 = Untersuchung erfolgte in Fremdlabor; #6 = unterliegt nicht der Akkreditierung
„<…" = Wert ist kleiner als die nebenstehende untere Grenze des Arbeitsbereiches

Figure C.10: Lab report of the nutritional analysis of the duck eggs of the experimental group (Experiment 2).

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DAkkS Deutsche Akkreditierungsstelle D-PL-14165-01-00

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Prüfbericht

Oldenburg, 29.08.2019
Seite 1 von 1
Berichts-Version: 1

Kunden-Nr.:	50178422	Probeneingang:	19.08.2019
Auftrags-Nr.:	1745198	Untersuchungsbeginn:	19.08.2019
Proben-Nr.:	**19FU033988**	Untersuchungsende:	29.08.2019
Probenart:	Eier & Eiprodukte		
Befindlich in:	Pappkarton		
Bezeichnung:	K 15.8.19		
Probenehmer:	durch Auftraggeber		

	Ergebnis in der Originalsubstanz
Rohfett A+B *VO (EG) 152 Anhang III, H; 2009*	**12,3 %**
Vitamin A *LUFA Nord-West 1/3-029; 2017-11*	**8840 IE/kg**
Vitamin E, berechnet als alpha-Tocopherolacetat *LUFA Nord-West 1/3-029; 2017-11*	**39 mg/kg**
Fettsäuremuster *ASU L 13.00-26; 2008-06 / ASU L 13.00-27/2; 2012-01*	**siehe Anlage**

Im Auftrag

Dr. Hartwig Wellmann
Laborbereichsleiter

Dieser Prüfbericht wurde maschinell erstellt und ist ohne Unterschrift gültig.

#2 = IfT, Oldenburg; #3 = IfL, Oldenburg; #4 = IfB, IfD, Hameln; #5 = Untersuchung erfolgte in Fremdlabor; #6 = unterliegt nicht der Akkreditierung
„<…" = Wert ist kleiner als die nebenstehende untere Grenze des Arbeitsbereiches

Die Untersuchungsergebnisse beziehen sich auf das uns vorliegende Probenmaterial. Dieser Prüfbericht darf nur vollständig und unverändert weiterverbreitet werden. Abweichende Vorgehensweisen bedürfen der schriftlichen Genehmigung der LUFA Nord-West. Die Akkreditierung gilt für den in der Urkundenanlage D-PL-14165-01-00 festgelegten Umfang.

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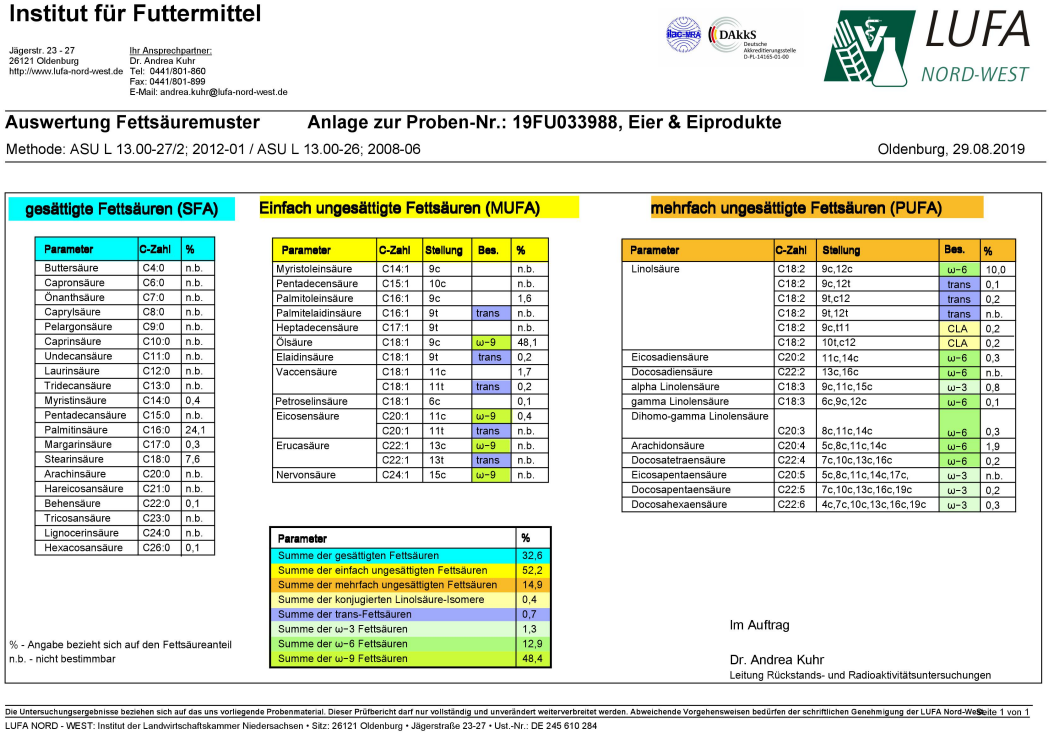
Figure C.11: Lab report of the nutritional analysis of the duck eggs of the control group (Experiment 3).



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LUFA NORD-WEST

Auswertung Fettsäuremuster **Anlage zur Proben-Nr.: 19FU033988, Eier & Eiprodukte**

Methode: ASU L 13.00-27/2; 2012-01 / ASU L 13.00-26; 2008-06 Oldenburg, 29.08.2019

gesättigte Fettsäuren (SFA)

Parameter	C-Zahl	%
Buttersäure	C4:0	n.b.
Capronsäure	C6:0	n.b.
Önanthsäure	C7:0	n.b.
Caprylsäure	C8:0	n.b.
Pelargonsäure	C9:0	n.b.
Caprinsäure	C10:0	n.b.
Undecansäure	C11:0	n.b.
Laurinsäure	C12:0	n.b.
Tridecansäure	C13:0	n.b.
Myristinsäure	C14:0	0,4
Pentadecansäure	C15:0	n.b.
Palmitinsäure	C16:0	24,1
Margarinsäure	C17:0	0,3
Stearinsäure	C18:0	7,6
Arachinsäure	C20:0	n.b.
Hareicosansäure	C21:0	n.b.
Behensäure	C22:0	0,1
Tricosansäure	C23:0	n.b.
Lignocerinsäure	C24:0	n.b.
Hexacosansäure	C26:0	0,1

Einfach ungesättigte Fettsäuren (MUFA)

Parameter	C-Zahl	Stellung	Bes.	%
Myristoleinsäure	C14:1	9c		n.b.
Pentadecensäure	C15:1	10c		n.b.
Palmitoleinsäure	C16:1	9c		1,6
Palmitelaidinsäure	C16:1	9t	trans	n.b.
Heptadecensäure	C17:1	9t		n.b.
Ölsäure	C18:1	9c	ω-9	48,1
Elaidinsäure	C18:1	9t	trans	0,2
Vaccensäure	C18:1	11c		1,7
	C18:1	11t	trans	0,2
Petroselinsäure	C18:1	6c		0,1
Eicosensäure	C20:1	11c	ω-9	0,4
	C20:1	11t	trans	n.b.
Erucasäure	C22:1	13c	ω-9	n.b.
	C22:1	13t	trans	n.b.
Nervonsäure	C24:1	15c	ω-9	n.b.

mehrfach ungesättigte Fettsäuren (PUFA)

Parameter	C-Zahl	Stellung	Bes.	%
Linolsäure	C18:2	9c,12c	ω-6	10,0
	C18:2	9c,12t	trans	0,1
	C18:2	9t,c12	trans	0,2
	C18:2	9t,12t	trans	n.b.
	C18:2	9c,t11	CLA	0,2
	C18:2	10t,c12	CLA	0,2
Eicosadiensäure	C20:2	11c,14c	ω-6	0,3
Docosadiensäure	C22:2	13c,16c	ω-6	n.b.
alpha Linolensäure	C18:3	9c,11c,15c	ω-3	0,8
gamma Linolensäure	C18:3	6c,9c,12c	ω-6	0,1
Dihomo-gamma Linolensäure	C20:3	8c,11c,14c	ω-6	0,3
Arachidonsäure	C20:4	5c,8c,11c,14c	ω-6	1,9
Docosatetraensäure	C22:4	7c,10c,13c,16c	ω-6	0,2
Eicosapentaensäure	C20:5	5c,8c,11c,14c,17c,	ω-3	n.b.
Docosapentaensäure	C22:5	7c,10c,13c,16c,19c	ω-3	0,2
Docosahexaensäure	C22:6	4c,7c,10c,13c,16c,19c	ω-3	0,3

Parameter	%
Summe der gesättigten Fettsäuren	32,6
Summe der einfach ungesättigten Fettsäuren	52,2
Summe der mehrfach ungesättigten Fettsäuren	14,9
Summe der konjugierten Linolsäure-Isomere	0,4
Summe der trans-Fettsäuren	0,7
Summe der ω-3 Fettsäuren	1,3
Summe der ω-6 Fettsäuren	12,9
Summe der ω-9 Fettsäuren	48,4

% - Angabe bezieht sich auf den Fettsäureanteil
n.b. - nicht bestimmbar

Im Auftrag

Dr. Andrea Kuhr
Leitung Rückstands- und Radioaktivitätsuntersuchungen

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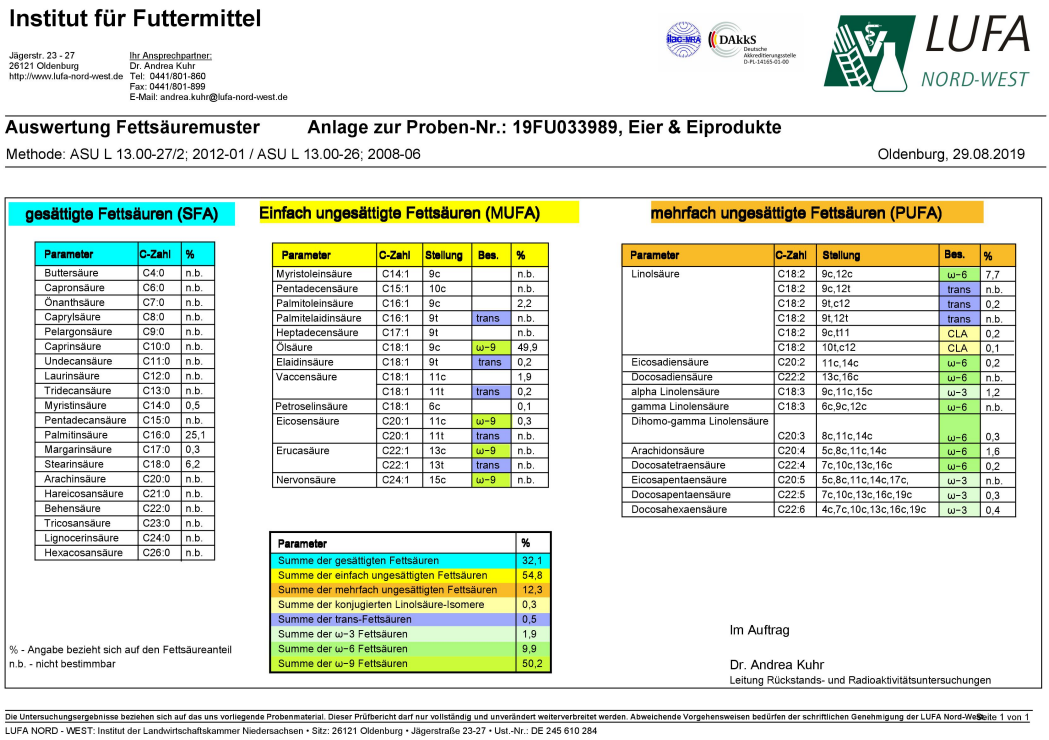
Figure C.12: Lab report of the FA profile of the duck eggs of the control group (Experiment 3).



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LUFA NORD-WEST

Auswertung Fettsäuremuster **Anlage zur Proben-Nr.: 19FU033989, Eier & Eiprodukte**

Methode: ASU L 13.00-27/2; 2012-01 / ASU L 13.00-26; 2008-06 Oldenburg, 29.08.2019

gesättigte Fettsäuren (SFA)

Parameter	C-Zahl	%
Buttersäure	C4:0	n.b.
Capronsäure	C6:0	n.b.
Önanthsäure	C7:0	n.b.
Caprylsäure	C8:0	n.b.
Pelargonsäure	C9:0	n.b.
Caprinsäure	C10:0	n.b.
Undecansäure	C11:0	n.b.
Laurinsäure	C12:0	n.b.
Tridecansäure	C13:0	n.b.
Myristinsäure	C14:0	0,5
Pentadecansäure	C15:0	n.b.
Palmitinsäure	C16:0	25,1
Margarinsäure	C17:0	0,3
Stearinsäure	C18:0	6,2
Arachinsäure	C20:0	n.b.
Hareicosansäure	C21:0	n.b.
Behensäure	C22:0	n.b.
Tricosansäure	C23:0	n.b.
Lignocerinsäure	C24:0	n.b.
Hexacosansäure	C26:0	n.b.

Einfach ungesättigte Fettsäuren (MUFA)

Parameter	C-Zahl	Stellung	Bes.	%
Myristoleinsäure	C14:1	9c		n.b.
Pentadecensäure	C15:1	10c		n.b.
Palmitoleinsäure	C16:1	9c		2,2
Palmitelaidinsäure	C16:1	9t	trans	n.b.
Heptadecensäure	C17:1	9t		n.b.
Ölsäure	C18:1	9c	ω-9	49,9
Elaidinsäure	C18:1	9t	trans	0,2
Vaccensäure	C18:1	11c		1,9
	C18:1	11t	trans	0,2
Petroselinsäure	C18:1	6c		0,1
Eicosensäure	C20:1	11c	ω-9	0,3
	C20:1	11t	trans	n.b.
Erucasäure	C22:1	13c	ω-9	n.b.
	C22:1	13t	trans	n.b.
Nervonsäure	C24:1	15c	ω-9	n.b.

mehrfach ungesättigte Fettsäuren (PUFA)

Parameter	C-Zahl	Stellung	Bes.	%
Linolsäure	C18:2	9c,12c	ω-6	7,7
	C18:2	9c,12t	trans	n.b.
	C18:2	9t,c12	trans	0,2
	C18:2	9t,12t	trans	n.b.
	C18:2	9c,t11	CLA	0,2
	C18:2	10t,c12	CLA	0,1
Eicosadiensäure	C20:2	11c,14c	ω-6	0,2
Docosadiensäure	C22:2	13c,16c	ω-6	n.b.
alpha Linolensäure	C18:3	9c,11c,15c	ω-3	1,2
gamma Linolensäure	C18:3	6c,9c,12c	ω-6	n.b.
Dihomo-gamma Linolensäure	C20:3	8c,11c,14c	ω-6	0,3
Arachidonsäure	C20:4	5c,8c,11c,14c	ω-6	1,6
Docosatetraensäure	C22:4	7c,10c,13c,16c	ω-6	0,2
Eicosapentaensäure	C20:5	5c,8c,11c,14c,17c,	ω-3	n.b.
Docosapentaensäure	C22:5	7c,10c,13c,16c,19c	ω-3	0,3
Docosahexaensäure	C22:6	4c,7c,10c,13c,16c,19c	ω-3	0,4

Parameter	%
Summe der gesättigten Fettsäuren	32,1
Summe der einfach ungesättigten Fettsäuren	54,8
Summe der mehrfach ungesättigten Fettsäuren	12,3
Summe der konjugierten Linolsäure-Isomere	0,3
Summe der trans-Fettsäuren	0,5
Summe der ω-3 Fettsäuren	1,9
Summe der ω-6 Fettsäuren	9,9
Summe der ω-9 Fettsäuren	50,2

% - Angabe bezieht sich auf den Fettsäureanteil
n.b. - nicht bestimmbar

Im Auftrag

Dr. Andrea Kuhr
Leitung Rückstands- und Radioaktivitätsuntersuchungen

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Figure C.13: Lab report of the FA profile of the duck eggs of the experimental group (Experiment 3).

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Prüfbericht

Oldenburg, 29.08.2019
Seite 1 von 1
Berichts-Version: 1

Kunden-Nr.:	50178422	Probeneingang:	19.08.2019
Auftrags-Nr.:	1745198	Untersuchungsbeginn:	19.08.2019
Proben-Nr.:	**19FU033989**	Untersuchungsende:	29.08.2019
Probenart:	Eier & Eiprodukte		
Befindlich in:	Pappkarton		
Bezeichnung:	V 15.8.19		
Probenehmer:	durch Auftraggeber		

	Ergebnis in der Originalsubstanz
Rohfett A+B *VO (EG) 152 Anhang III, H; 2009*	**15,1 %**
Vitamin A *LUFA Nord-West 1/3-029; 2017-11*	**10000 IE/kg**
Vitamin E, berechnet als alpha-Tocopherolacetat *LUFA Nord-West 1/3-029; 2017-11*	**47 mg/kg**
Fettsäuremuster *ASU L 13.00-26; 2008-06 / ASU L 13.00-27/2; 2012-01*	**siehe Anlage**

Im Auftrag

Dr. Hartwig Wellmann
Laborbereichsleiter

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„<…" = Wert ist kleiner als die nebenstehende untere Grenze des Arbeitsbereiches

Die Untersuchungsergebnisse beziehen sich auf das uns vorliegende Probenmaterial. Dieser Prüfbericht darf nur vollständig und unverändert weiterverbreitet werden. Abweichende Vorgehensweisen bedürfen der schriftlichen Genehmigung der LUFA Nord-West. Die Akkreditierung gilt für den in der Urkundenanlage D-PL-14165-01-00 festgelegten Umfang.

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Figure C.14: Lab report of the nutritional analysis of the duck eggs of the experimental group (Experiment 3).

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Prüfbericht

Oldenburg, 05.12.2019
Seite 1 von 1
Berichts-Version: 1

Kunden-Nr.:	50178422	Probeneingang:	05.11.2019
Auftrags-Nr.:	1850734	Untersuchungsbeginn:	05.11.2019
Proben-Nr.:	**19FU045719**	Untersuchungsende:	05.12.2019
Probenart:	Eier & Eiprodukte		
Befindlich in:	Pappkarton		
Bezeichnung:	V 25.09.2019		
Probenehmer:	durch Auftraggeber		
Probenzustand:	Probeneingang gekühlt		

	Ergebnis in der Originalsubstanz
Rohfett A+B *VO (EG) 152 Anhang III, H; 2009*	**10,6 %**
Vitamin A *LUFA Nord-West 1/3-029; 2017-11*	**9300 IE/kg**
Vitamin E, berechnet als alpha-Tocopherolacetat *LUFA Nord-West 1/3-029; 2017-11*	**34 mg/kg**
Fettsäuremuster *ASU L 13.00-26; 2008-06 / ASU L 13.00-27/2; 2012-01*	**siehe Anlage**

Im Auftrag

Dr. Michael Egert
Institutsleiter

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„<…" = Wert ist kleiner als die nebenstehende untere Grenze des Arbeitsbereiches

Die Untersuchungsergebnisse beziehen sich auf das uns vorliegende Probenmaterial. Dieser Prüfbericht darf nur vollständig und unverändert weiterverbreitet werden. Abweichende Vorgehensweisen bedürfen der schriftlichen Genehmigung der LUFA Nord-West. Die Akkreditierung gilt für den in der Urkundenanlage D-PL-14165-01-00 festgelegten Umfang.

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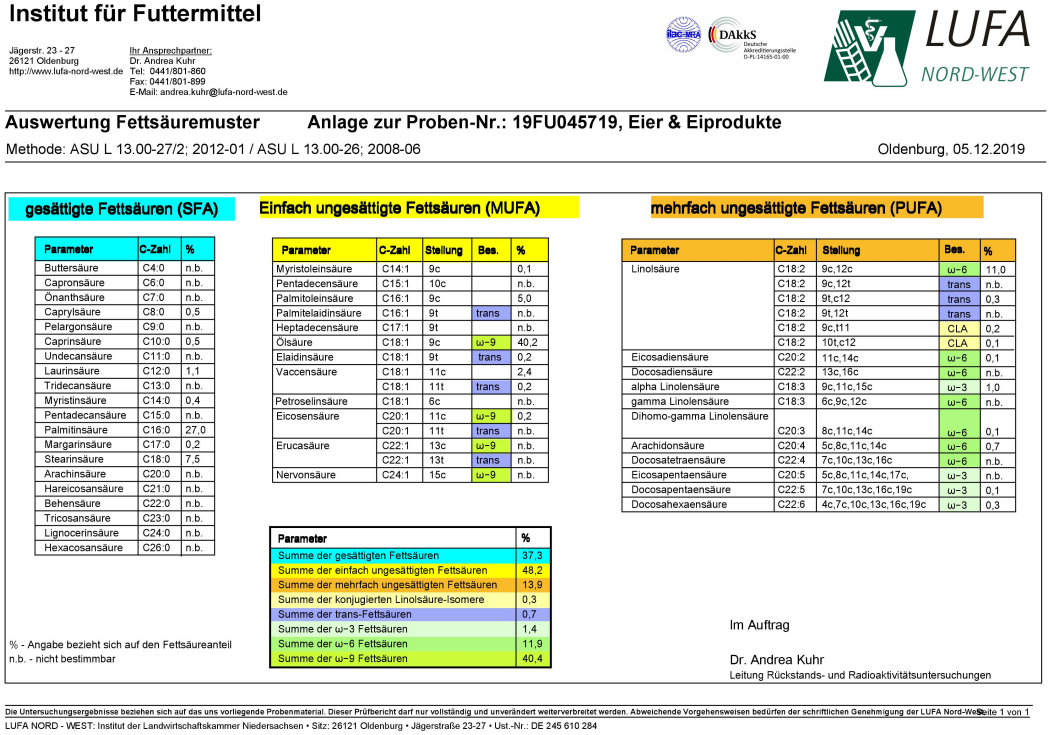
Figure C.15: Lab report of the nutritional analysis of the chicken eggs of the control group (Experiment 4).



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LUFA NORD-WEST

Auswertung Fettsäuremuster **Anlage zur Proben-Nr.: 19FU045719, Eier & Eiprodukte**

Methode: ASU L 13.00-27/2; 2012-01 / ASU L 13.00-26; 2008-06 Oldenburg, 05.12.2019

gesättigte Fettsäuren (SFA)

Parameter	C-Zahl	%
Buttersäure	C4:0	n.b.
Capronsäure	C6:0	n.b.
Önanthsäure	C7:0	n.b.
Caprylsäure	C8:0	0,5
Pelargonsäure	C9:0	n.b.
Caprinsäure	C10:0	0,5
Undecansäure	C11:0	n.b.
Laurinsäure	C12:0	1,1
Tridecansäure	C13:0	n.b.
Myristinsäure	C14:0	0,4
Pentadecansäure	C15:0	n.b.
Palmitinsäure	C16:0	27,0
Margarinsäure	C17:0	0,2
Stearinsäure	C18:0	7,5
Arachinsäure	C20:0	n.b.
Hareicosansäure	C21:0	n.b.
Behensäure	C22:0	n.b.
Tricosansäure	C23:0	n.b.
Lignocerinsäure	C24:0	n.b.
Hexacosansäure	C26:0	n.b.

Einfach ungesättigte Fettsäuren (MUFA)

Parameter	C-Zahl	Stellung	Bes.	%
Myristoleinsäure	C14:1	9c		0,1
Pentadecensäure	C15:1	10c		n.b.
Palmitoleinsäure	C16:1	9c		5,0
Palmitelaidinsäure	C16:1	9t	trans	n.b.
Heptadecensäure	C17:1	9t		n.b.
Ölsäure	C18:1	9c	ω-9	40,2
Elaidinsäure	C18:1	9t	trans	0,2
Vaccensäure	C18:1	11c		2,4
	C18:1	11t	trans	0,2
Petroselinsäure	C18:1	6c		n.b.
Eicosensäure	C20:1	11c	ω-9	0,2
	C20:1	11t	trans	n.b.
Erucasäure	C22:1	13c	ω-9	n.b.
	C22:1	13t	trans	n.b.
Nervonsäure	C24:1	15c	ω-9	n.b.

mehrfach ungesättigte Fettsäuren (PUFA)

Parameter	C-Zahl	Stellung	Bes.	%
Linolsäure	C18:2	9c,12c	ω-6	11,0
	C18:2	9c,12t	trans	n.b.
	C18:2	9t,c12	trans	0,3
	C18:2	9t,12t	trans	n.b.
	C18:2	9c,t11	CLA	0,2
	C18:2	10t,c12	CLA	0,1
Eicosadiensäure	C20:2	11c,14c	ω-6	0,1
Docosadiensäure	C22:2	13c,16c	ω-6	n.b.
alpha Linolensäure	C18:3	9c,11c,15c	ω-3	1,0
gamma Linolensäure	C18:3	6c,9c,12c	ω-6	n.b.
Dihomo-gamma Linolensäure	C20:3	8c,11c,14c	ω-6	0,1
Arachidonsäure	C20:4	5c,8c,11c,14c	ω-6	0,7
Docosatetraensäure	C22:4	7c,10c,13c,16c	ω-6	n.b.
Eicosapentaensäure	C20:5	5c,8c,11c,14c,17c,	ω-3	n.b.
Docosapentaensäure	C22:5	7c,10c,13c,16c,19c	ω-3	0,1
Docosahexaensäure	C22:6	4c,7c,10c,13c,16c,19c	ω-3	0,3

Parameter	%
Summe der gesättigten Fettsäuren	37,3
Summe der einfach ungesättigten Fettsäuren	48,2
Summe der mehrfach ungesättigten Fettsäuren	13,9
Summe der konjugierten Linolsäure-Isomere	0,3
Summe der trans-Fettsäuren	0,7
Summe der ω-3 Fettsäuren	1,4
Summe der ω-6 Fettsäuren	11,9
Summe der ω-9 Fettsäuren	40,4

% - Angabe bezieht sich auf den Fettsäureanteil
n.b. - nicht bestimmbar

Im Auftrag

Dr. Andrea Kuhr
Leitung Rückstands- und Radioaktivitätsuntersuchungen

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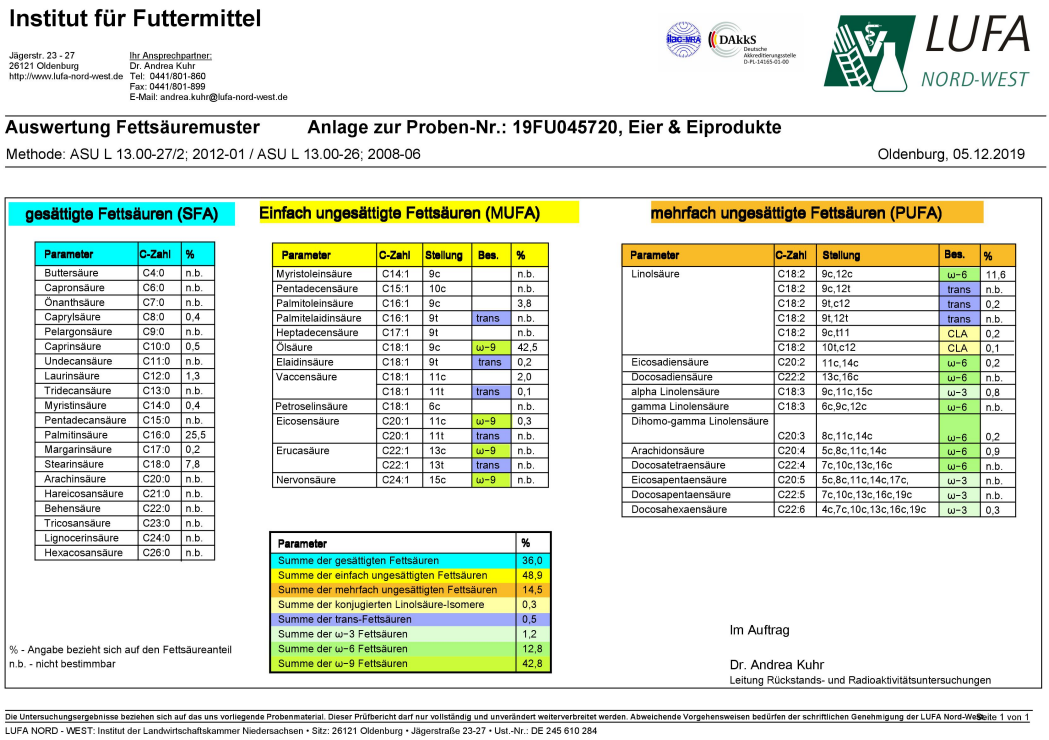
Figure C.16: Lab report of the FA profile of the chicken eggs of the control group (Experiment 4).



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Auswertung Fettsäuremuster **Anlage zur Proben-Nr.: 19FU045720, Eier & Eiprodukte**

Methode: ASU L 13.00-27/2; 2012-01 / ASU L 13.00-26; 2008-06 Oldenburg, 05.12.2019

gesättigte Fettsäuren (SFA)

Parameter	C-Zahl	%
Buttersäure	C4:0	n.b.
Capronsäure	C6:0	n.b.
Önanthsäure	C7:0	n.b.
Caprylsäure	C8:0	0,4
Pelargonsäure	C9:0	n.b.
Caprinsäure	C10:0	0,5
Undecansäure	C11:0	n.b.
Laurinsäure	C12:0	1,3
Tridecansäure	C13:0	n.b.
Myristinsäure	C14:0	0,4
Pentadecansäure	C15:0	n.b.
Palmitinsäure	C16:0	25,5
Margarinsäure	C17:0	0,2
Stearinsäure	C18:0	7,8
Arachinsäure	C20:0	n.b.
Hareicosansäure	C21:0	n.b.
Behensäure	C22:0	n.b.
Tricosansäure	C23:0	n.b.
Lignocerinsäure	C24:0	n.b.
Hexacosansäure	C26:0	n.b.

Einfach ungesättigte Fettsäuren (MUFA)

Parameter	C-Zahl	Stellung	Bes.	%
Myristoleinsäure	C14:1	9c		n.b.
Pentadecensäure	C15:1	10c		n.b.
Palmitoleinsäure	C16:1	9c		3,8
Palmitelaidinsäure	C16:1	9t	trans	n.b.
Heptadecensäure	C17:1	9t		n.b.
Ölsäure	C18:1	9c	ω-9	42,5
Elaidinsäure	C18:1	9t	trans	0,2
Vaccensäure	C18:1	11c		2,0
	C18:1	11t	trans	0,1
Petroselinsäure	C18:1	6c		n.b.
Eicosensäure	C20:1	11c	ω-9	0,3
	C20:1	11t	trans	n.b.
Erucasäure	C22:1	13c	ω-9	n.b.
	C22:1	13t	trans	n.b.
Nervonsäure	C24:1	15c	ω-9	n.b.

mehrfach ungesättigte Fettsäuren (PUFA)

Parameter	C-Zahl	Stellung	Bes.	%
Linolsäure	C18:2	9c,12c	ω-6	11,6
	C18:2	9c,12t	trans	n.b.
	C18:2	9t,c12	trans	0,2
	C18:2	9t,12t	trans	n.b.
	C18:2	9c,t11	CLA	0,2
	C18:2	10t,c12	CLA	0,1
Eicosadiensäure	C20:2	11c,14c	ω-6	0,2
Docosadiensäure	C22:2	13c,16c	ω-6	n.b.
alpha Linolensäure	C18:3	9c,11c,15c	ω-3	0,8
gamma Linolensäure	C18:3	6c,9c,12c	ω-6	n.b.
Dihomo-gamma Linolensäure	C20:3	8c,11c,14c	ω-6	0,2
Arachidonsäure	C20:4	5c,8c,11c,14c	ω-6	0,9
Docosatetraensäure	C22:4	7c,10c,13c,16c	ω-6	n.b.
Eicosapentaensäure	C20:5	5c,8c,11c,14c,17c,	ω-3	n.b.
Docosapentaensäure	C22:5	7c,10c,13c,16c,19c	ω-3	n.b.
Docosahexaensäure	C22:6	4c,7c,10c,13c,16c,19c	ω-3	0,3

Parameter	%
Summe der gesättigten Fettsäuren	36,0
Summe der einfach ungesättigten Fettsäuren	48,9
Summe der mehrfach ungesättigten Fettsäuren	14,5
Summe der konjugierten Linolsäure-Isomere	0,3
Summe der trans-Fettsäuren	0,5
Summe der ω-3 Fettsäuren	1,2
Summe der ω-6 Fettsäuren	12,8
Summe der ω-9 Fettsäuren	42,8

% - Angabe bezieht sich auf den Fettsäureanteil
n.b. - nicht bestimmbar

Im Auftrag

Dr. Andrea Kuhr
Leitung Rückstands- und Radioaktivitätsuntersuchungen

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Figure C.17: Lab report of the FA profile of the chicken eggs of the experimental group (Experiment 4).

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Prüfbericht

Oldenburg, 05.12.2019
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Berichts-Version: 1

Kunden-Nr.:	50178422	Probeneingang:	05.11.2019
Auftrags-Nr.:	1850734	Untersuchungsbeginn:	05.11.2019
Proben-Nr.:	**19FU045720**	Untersuchungsende:	05.12.2019
Probenart:	Eier & Eiprodukte		
Befindlich in:	Pappkarton		
Bezeichnung:	K 25.09.2019		
Probenehmer:	durch Auftraggeber		
Probenzustand:	Probeneingang gekühlt		

	Ergebnis in der Originalsubstanz
Rohfett A+B *VO (EG) 152 Anhang III, H; 2009*	**12,3 %**
Vitamin A *LUFA Nord-West 1/3-029; 2017-11*	**10000 IE/kg**
Vitamin E, berechnet als alpha-Tocopherolacetat *LUFA Nord-West 1/3-029; 2017-11*	**57 mg/kg**
Fettsäuremuster *ASU L 13.00-26; 2008-06 / ASU L 13.00-27/2; 2012-01*	**siehe Anlage**

Im Auftrag

Dr. Michael Egert
Institutsleiter

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Figure C.18: Lab report of the nutritional analysis of the chicken eggs of the experimental group (Experiment 4).

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Prüfbericht

Oldenburg, 05.12.2019
Seite 1 von 1
Berichts-Version: 1

Kunden-Nr.:	50178422	Probeneingang:	05.11.2019
Auftrags-Nr.:	1850734	Untersuchungsbeginn:	05.11.2019
Proben-Nr.:	**19FU045722**	Untersuchungsende:	05.12.2019
Probenart:	Eier & Eiprodukte		
Befindlich in:	Pappkarton		
Bezeichnung:	K 14.10.2019		
Probenehmer:	durch Auftraggeber		
Probenzustand:	Probeneingang gekühlt		

	Ergebnis in der Originalsubstanz
Rohfett A+B *VO (EG) 152 Anhang III, H; 2009*	**10,8 %**
Vitamin A *LUFA Nord-West 1/3-029; 2017-11*	**8200 IE/kg**
Vitamin E, berechnet als alpha-Tocopherolacetat *LUFA Nord-West 1/3-029; 2017-11*	**40 mg/kg**
Fettsäuremuster *ASU L 13.00-26; 2008-06 / ASU L 13.00-27/2; 2012-01*	**siehe Anlage**

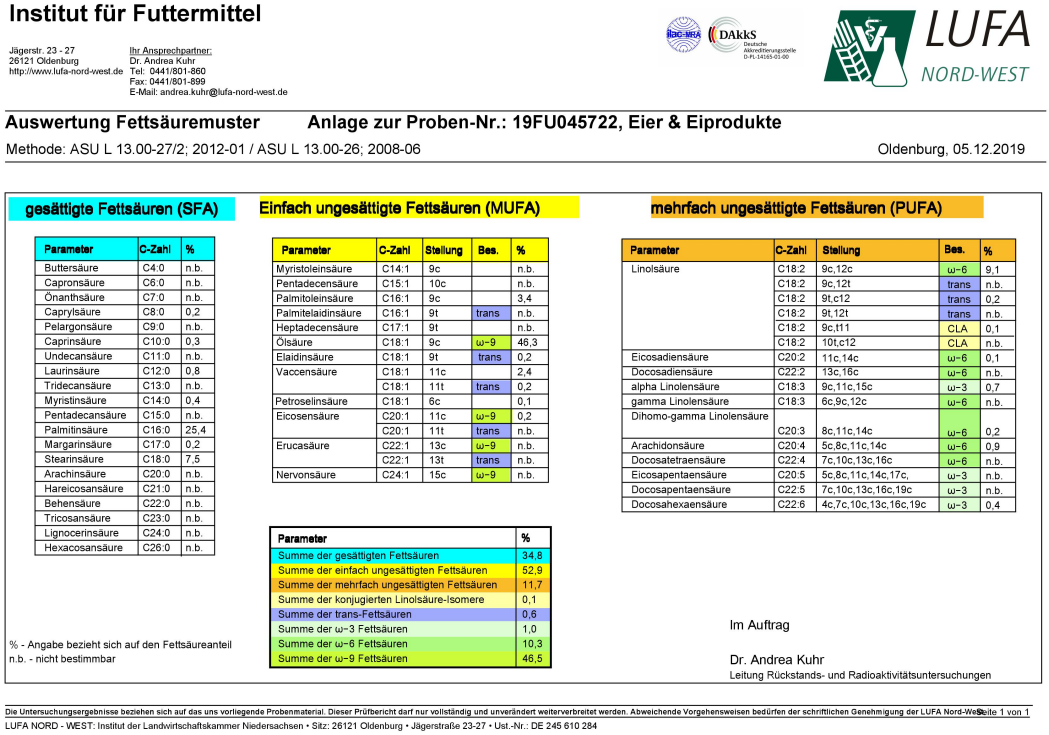
Im Auftrag

Dr. Michael Egert
Institutsleiter

Dieser Prüfbericht wurde maschinell erstellt und ist ohne Unterschrift gültig.

#2 = IfT, Oldenburg; #3 = IfL, Oldenburg; #4 = IfB, IfD, Hameln; #5 = Untersuchung erfolgte in Fremdlabor; #6 = unterliegt nicht der Akkreditierung
„<…" = Wert ist kleiner als die nebenstehende untere Grenze des Arbeitsbereiches

Figure C.19: Lab report of the nutritional analysis of the chicken eggs of the control group (Experiment 5).



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LUFA NORD-WEST

Auswertung Fettsäuremuster **Anlage zur Proben-Nr.: 19FU045722, Eier & Eiprodukte**

Methode: ASU L 13.00-27/2; 2012-01 / ASU L 13.00-26; 2008-06 Oldenburg, 05.12.2019

gesättigte Fettsäuren (SFA)

Parameter	C-Zahl	%
Buttersäure	C4:0	n.b.
Capronsäure	C6:0	n.b.
Önanthsäure	C7:0	n.b.
Caprylsäure	C8:0	0,2
Pelargonsäure	C9:0	n.b.
Caprinsäure	C10:0	0,3
Undecansäure	C11:0	n.b.
Laurinsäure	C12:0	0,8
Tridecansäure	C13:0	n.b.
Myristinsäure	C14:0	0,4
Pentadecansäure	C15:0	n.b.
Palmitinsäure	C16:0	25,4
Margarinsäure	C17:0	0,2
Stearinsäure	C18:0	7,5
Arachinsäure	C20:0	n.b.
Hareicosansäure	C21:0	n.b.
Behensäure	C22:0	n.b.
Tricosansäure	C23:0	n.b.
Lignocerinsäure	C24:0	n.b.
Hexacosansäure	C26:0	n.b.

Einfach ungesättigte Fettsäuren (MUFA)

Parameter	C-Zahl	Stellung	Bes.	%
Myristoleinsäure	C14:1	9c		n.b.
Pentadecensäure	C15:1	10c		n.b.
Palmitoleinsäure	C16:1	9c		3,4
Palmitelaidinsäure	C16:1	9t	trans	n.b.
Heptadecensäure	C17:1	9t		n.b.
Ölsäure	C18:1	9c	ω-9	46,3
Elaidinsäure	C18:1	9t	trans	0,2
Vaccensäure	C18:1	11c		2,4
	C18:1	11t	trans	0,2
Petroselinsäure	C18:1	6c		0,1
Eicosensäure	C20:1	11c	ω-9	0,2
	C20:1	11t	trans	n.b.
Erucasäure	C22:1	13c	ω-9	n.b.
	C22:1	13t	trans	n.b.
Nervonsäure	C24:1	15c	ω-9	n.b.

mehrfach ungesättigte Fettsäuren (PUFA)

Parameter	C-Zahl	Stellung	Bes.	%
Linolsäure	C18:2	9c,12c	ω-6	9,1
	C18:2	9c,12t	trans	n.b.
	C18:2	9t,c12	trans	0,2
	C18:2	9t,12t	trans	n.b.
	C18:2	9c,t11	CLA	0,1
	C18:2	10t,c12	CLA	n.b.
Eicosadiensäure	C20:2	11c,14c	ω-6	0,1
Docosadiensäure	C22:2	13c,16c	ω-6	n.b.
alpha Linolensäure	C18:3	9c,11c,15c	ω-3	0,7
gamma Linolensäure	C18:3	6c,9c,12c	ω-6	n.b.
Dihomo-gamma Linolensäure	C20:3	8c,11c,14c	ω-6	0,2
Arachidonsäure	C20:4	5c,8c,11c,14c	ω-6	0,9
Docosatetraensäure	C22:4	7c,10c,13c,16c	ω-6	n.b.
Eicosapentaensäure	C20:5	5c,8c,11c,14c,17c,	ω-3	n.b.
Docosapentaensäure	C22:5	7c,10c,13c,16c,19c	ω-3	n.b.
Docosahexaensäure	C22:6	4c,7c,10c,13c,16c,19c	ω-3	0,4

Parameter	%
Summe der gesättigten Fettsäuren	34,8
Summe der einfach ungesättigten Fettsäuren	52,9
Summe der mehrfach ungesättigten Fettsäuren	11,7
Summe der konjugierten Linolsäure-Isomere	0,1
Summe der trans-Fettsäuren	0,6
Summe der ω-3 Fettsäuren	1,0
Summe der ω-6 Fettsäuren	10,3
Summe der ω-9 Fettsäuren	46,5

% - Angabe bezieht sich auf den Fettsäureanteil
n.b. - nicht bestimmbar

Im Auftrag

Dr. Andrea Kuhr
Leitung Rückstands- und Radioaktivitätsuntersuchungen

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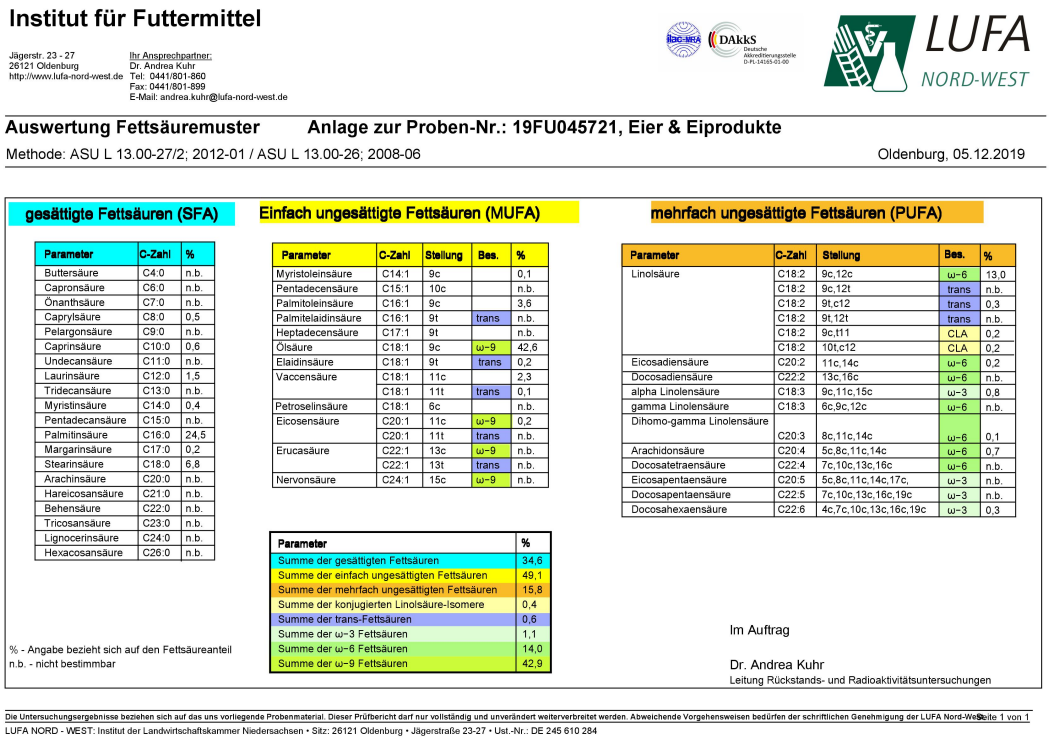
Figure C.20: Lab report of the FA profile of the chicken eggs of the control group (Experiment 5).



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LUFA NORD-WEST

Auswertung Fettsäuremuster **Anlage zur Proben-Nr.: 19FU045721, Eier & Eiprodukte**

Methode: ASU L 13.00-27/2; 2012-01 / ASU L 13.00-26; 2008-06 Oldenburg, 05.12.2019

gesättigte Fettsäuren (SFA)

Parameter	C-Zahl	%
Buttersäure	C4:0	n.b.
Capronsäure	C6:0	n.b.
Önanthsäure	C7:0	n.b.
Caprylsäure	C8:0	0,5
Pelargonsäure	C9:0	n.b.
Caprinsäure	C10:0	0,6
Undecansäure	C11:0	n.b.
Laurinsäure	C12:0	1,5
Tridecansäure	C13:0	n.b.
Myristinsäure	C14:0	0,4
Pentadecansäure	C15:0	n.b.
Palmitinsäure	C16:0	24,5
Margarinsäure	C17:0	0,2
Stearinsäure	C18:0	6,8
Arachinsäure	C20:0	n.b.
Hareicosansäure	C21:0	n.b.
Behensäure	C22:0	n.b.
Tricosansäure	C23:0	n.b.
Lignocerinsäure	C24:0	n.b.
Hexacosansäure	C26:0	n.b.

Einfach ungesättigte Fettsäuren (MUFA)

Parameter	C-Zahl	Stellung	Bes.	%
Myristoleinsäure	C14:1	9c		0,1
Pentadecensäure	C15:1	10c		n.b.
Palmitoleinsäure	C16:1	9c		3,6
Palmitelaidinsäure	C16:1	9t	trans	n.b.
Heptadecensäure	C17:1	9t		n.b.
Ölsäure	C18:1	9c	ω-9	42,6
Elaidinsäure	C18:1	9t	trans	0,2
Vaccensäure	C18:1	11c		2,3
	C18:1	11t	trans	0,1
Petroselinsäure	C18:1	6c		n.b.
Eicosensäure	C20:1	11c	ω-9	0,2
	C20:1	11t	trans	n.b.
Erucasäure	C22:1	13c	ω-9	n.b.
	C22:1	13t	trans	n.b.
Nervonsäure	C24:1	15c	ω-9	n.b.

mehrfach ungesättigte Fettsäuren (PUFA)

Parameter	C-Zahl	Stellung	Bes.	%
Linolsäure	C18:2	9c,12c	ω-6	13,0
	C18:2	9c,12t	trans	n.b.
	C18:2	9t,c12	trans	0,3
	C18:2	9t,12t	trans	n.b.
	C18:2	9c,t11	CLA	0,2
	C18:2	10t,c12	CLA	0,2
Eicosadiensäure	C20:2	11c,14c	ω-6	0,2
Docosadiensäure	C22:2	13c,16c	ω-6	n.b.
alpha Linolensäure	C18:3	9c,11c,15c	ω-3	0,8
gamma Linolensäure	C18:3	6c,9c,12c	ω-6	n.b.
Dihomo-gamma Linolensäure	C20:3	8c,11c,14c	ω-6	0,1
Arachidonsäure	C20:4	5c,8c,11c,14c	ω-6	0,7
Docosatetraensäure	C22:4	7c,10c,13c,16c	ω-6	n.b.
Eicosapentaensäure	C20:5	5c,8c,11c,14c,17c,	ω-3	n.b.
Docosapentaensäure	C22:5	7c,10c,13c,16c,19c	ω-3	n.b.
Docosahexaensäure	C22:6	4c,7c,10c,13c,16c,19c	ω-3	0,3

Parameter	%
Summe der gesättigten Fettsäuren	34,6
Summe der einfach ungesättigten Fettsäuren	49,1
Summe der mehrfach ungesättigten Fettsäuren	15,8
Summe der konjugierten Linolsäure-Isomere	0,4
Summe der trans-Fettsäuren	0,6
Summe der ω-3 Fettsäuren	1,1
Summe der ω-6 Fettsäuren	14,0
Summe der ω-9 Fettsäuren	42,9

% - Angabe bezieht sich auf den Fettsäureanteil
n.b. - nicht bestimmbar

Im Auftrag

Dr. Andrea Kuhr
Leitung Rückstands- und Radioaktivitätsuntersuchungen

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Figure C.21: Lab report of the FA profile of the chicken eggs of the experimental group (Experiment 5).

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Prüfbericht

Oldenburg, 05.12.2019
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Kunden-Nr.:	50178422	Probeneingang:	05.11.2019
Auftrags-Nr.:	1850734	Untersuchungsbeginn:	05.11.2019
Proben-Nr.:	**19FU045721**	Untersuchungsende:	05.12.2019
Probenart:	Eier & Eiprodukte		
Befindlich in:	Pappkarton		
Bezeichnung:	V 14.10.2019		
Probenehmer:	durch Auftraggeber		
Probenzustand:	Probeneingang gekühlt		

	Ergebnis in der Originalsubstanz
Rohfett A+B *VO (EG) 152 Anhang III, H; 2009*	**10,9 %**
Vitamin A *LUFA Nord-West 1/3-029; 2017-11*	**9100 IE/kg**
Vitamin E, berechnet als alpha-Tocopherolacetat *LUFA Nord-West 1/3-029; 2017-11*	**42 mg/kg**
Fettsäuremuster *ASU L 13.00-26; 2008-06 / ASU L 13.00-27/2; 2012-01*	**siehe Anlage**

Im Auftrag

Dr. Michael Egert
Institutsleiter

Dieser Prüfbericht wurde maschinell erstellt und ist ohne Unterschrift gültig.

#2 = IfT, Oldenburg; #3 = IfL, Oldenburg; #4 = IfB, IfD, Hameln; #5 = Untersuchung erfolgte in Fremdlabor; #6 = unterliegt nicht der Akkreditierung
„<…" = Wert ist kleiner als die nebenstehende untere Grenze des Arbeitsbereiches

Die Untersuchungsergebnisse beziehen sich auf das uns vorliegende Probenmaterial. Dieser Prüfbericht darf nur vollständig und unverändert weiterverbreitet werden. Abweichende Vorgehensweisen bedürfen der schriftlichen Genehmigung der LUFA Nord-West. Die Akkreditierung gilt für den in der Urkundenanlage D-PL-14165-01-00 festgelegten Umfang.

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Figure C.22: Lab report of the nutritional analysis of the chicken eggs of the experimental group (Experiment 5).

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Prüfbericht

Oldenburg, 05.12.2019
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Kunden-Nr.:	50178422	Probeneingang:	05.11.2019
Auftrags-Nr.:	1850734	Untersuchungsbeginn:	05.11.2019
Proben-Nr.:	**19FU045724**	Untersuchungsende:	05.12.2019
Probenart:	Eier & Eiprodukte		
Befindlich in:	Pappkarton		
Bezeichnung:	K 01.11.2019		
Probenehmer:	durch Auftraggeber		
Probenzustand:	Probeneingang gekühlt		

	Ergebnis in der Originalsubstanz
Rohfett A+B *VO (EG) 152 Anhang III, H; 2009*	**11,6 %**
Vitamin A *LUFA Nord-West 1/3-029; 2017-11*	**8500 IE/kg**
Vitamin E, berechnet als alpha-Tocopherolacetat *LUFA Nord-West 1/3-029; 2017-11*	**40 mg/kg**
Fettsäuremuster *ASU L 13.00-26; 2008-06 / ASU L 13.00-27/2; 2012-01*	**siehe Anlage**

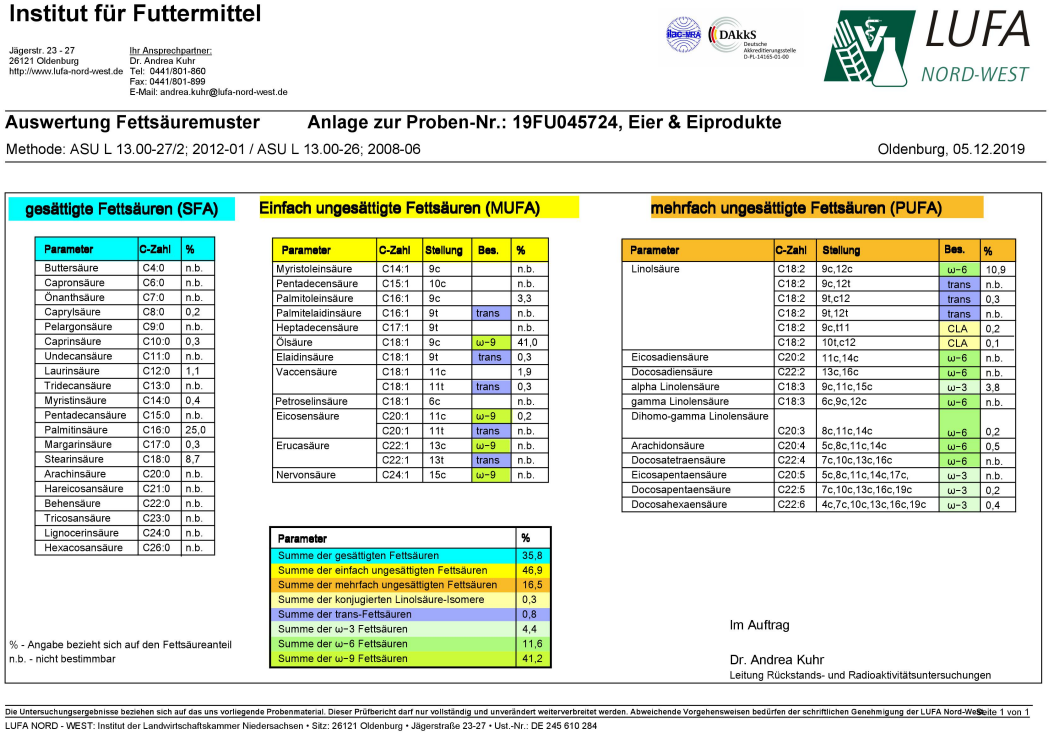
Im Auftrag

Dr. Michael Egert
Institutsleiter

Dieser Prüfbericht wurde maschinell erstellt und ist ohne Unterschrift gültig.

#2 = IfT, Oldenburg; #3 = IfL, Oldenburg; #4 = IfB, IfD, Hameln; #5 = Untersuchung erfolgte in Fremdlabor; #6 = unterliegt nicht der Akkreditierung
„<…" = Wert ist kleiner als die nebenstehende untere Grenze des Arbeitsbereiches

Figure C.23: Lab report of the nutritional analysis of the chicken eggs of the control group (Experiment 6).



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LUFA NORD-WEST

Auswertung Fettsäuremuster **Anlage zur Proben-Nr.: 19FU045724, Eier & Eiprodukte**

Methode: ASU L 13.00-27/2; 2012-01 / ASU L 13.00-26; 2008-06 Oldenburg, 05.12.2019

gesättigte Fettsäuren (SFA)

Parameter	C-Zahl	%
Buttersäure	C4:0	n.b.
Capronsäure	C6:0	n.b.
Önanthsäure	C7:0	n.b.
Caprylsäure	C8:0	0,2
Pelargonsäure	C9:0	n.b.
Caprinsäure	C10:0	0,3
Undecansäure	C11:0	n.b.
Laurinsäure	C12:0	1,1
Tridecansäure	C13:0	n.b.
Myristinsäure	C14:0	0,4
Pentadecansäure	C15:0	n.b.
Palmitinsäure	C16:0	25,0
Margarinsäure	C17:0	0,3
Stearinsäure	C18:0	8,7
Arachinsäure	C20:0	n.b.
Hareicosansäure	C21:0	n.b.
Behensäure	C22:0	n.b.
Tricosansäure	C23:0	n.b.
Lignocerinsäure	C24:0	n.b.
Hexacosansäure	C26:0	n.b.

Einfach ungesättigte Fettsäuren (MUFA)

Parameter	C-Zahl	Stellung	Bes.	%
Myristoleinsäure	C14:1	9c		n.b.
Pentadecensäure	C15:1	10c		n.b.
Palmitoleinsäure	C16:1	9c		3,3
Palmitelaidinsäure	C16:1	9t	trans	n.b.
Heptadecensäure	C17:1	9t		n.b.
Ölsäure	C18:1	9c	ω-9	41,0
Elaidinsäure	C18:1	9t	trans	0,3
Vaccensäure	C18:1	11c		1,9
	C18:1	11t	trans	0,3
Petroselinsäure	C18:1	6c		n.b.
Eicosensäure	C20:1	11c	ω-9	0,2
	C20:1	11t	trans	n.b.
Erucasäure	C22:1	13c	ω-9	n.b.
	C22:1	13t	trans	n.b.
Nervonsäure	C24:1	15c	ω-9	n.b.

mehrfach ungesättigte Fettsäuren (PUFA)

Parameter	C-Zahl	Stellung	Bes.	%
Linolsäure	C18:2	9c,12c	ω-6	10,9
	C18:2	9c,12t	trans	n.b.
	C18:2	9t,c12	trans	0,3
	C18:2	9t,12t	trans	n.b.
	C18:2	9c,t11	CLA	0,2
	C18:2	10t,c12	CLA	0,1
Eicosadiensäure	C20:2	11c,14c	ω-6	n.b.
Docosadiensäure	C22:2	13c,16c	ω-6	n.b.
alpha Linolensäure	C18:3	9c,11c,15c	ω-3	3,8
gamma Linolensäure	C18:3	6c,9c,12c	ω-6	n.b.
Dihomo-gamma Linolensäure	C20:3	8c,11c,14c	ω-6	0,2
Arachidonsäure	C20:4	5c,8c,11c,14c	ω-6	0,5
Docosatetraensäure	C22:4	7c,10c,13c,16c	ω-6	n.b.
Eicosapentaensäure	C20:5	5c,8c,11c,14c,17c,	ω-3	n.b.
Docosapentaensäure	C22:5	7c,10c,13c,16c,19c	ω-3	0,2
Docosahexaensäure	C22:6	4c,7c,10c,13c,16c,19c	ω-3	0,4

Parameter	%
Summe der gesättigten Fettsäuren	35,8
Summe der einfach ungesättigten Fettsäuren	46,9
Summe der mehrfach ungesättigten Fettsäuren	16,5
Summe der konjugierten Linolsäure-Isomere	0,3
Summe der trans-Fettsäuren	0,8
Summe der ω-3 Fettsäuren	4,4
Summe der ω-6 Fettsäuren	11,6
Summe der ω-9 Fettsäuren	41,2

% - Angabe bezieht sich auf den Fettsäureanteil
n.b. - nicht bestimmbar

Im Auftrag

Dr. Andrea Kuhr
Leitung Rückstands- und Radioaktivitätsuntersuchungen

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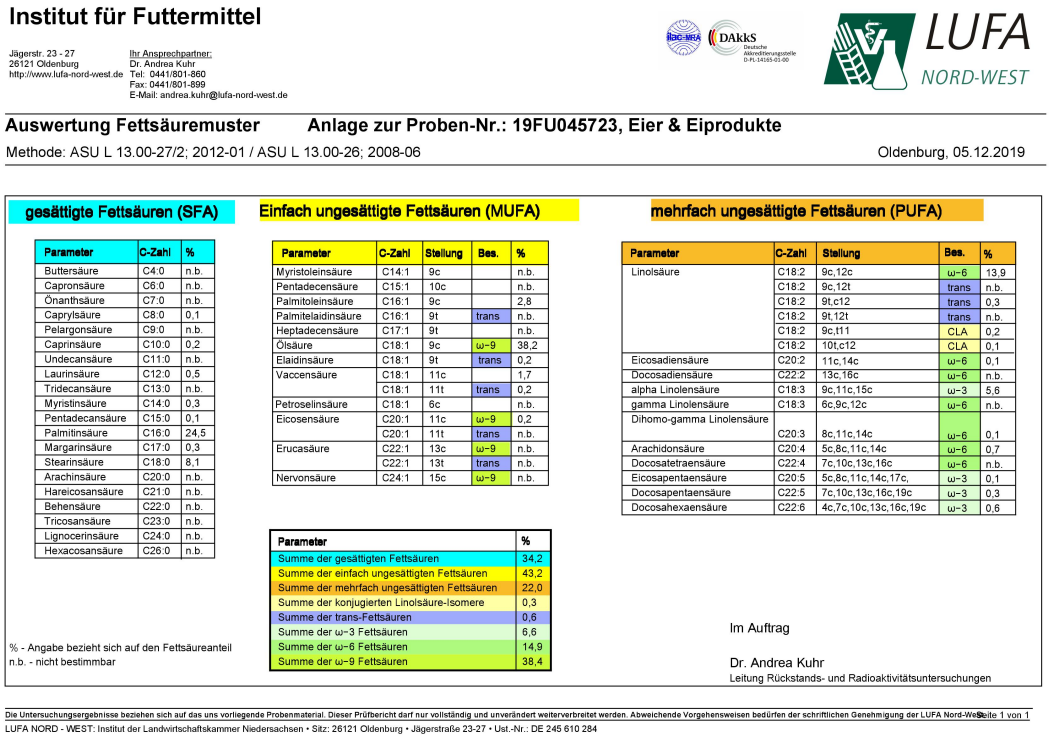
Figure C.24: Lab report of the FA profile of the chicken eggs of the control group (Experiment 6).



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LUFA NORD-WEST

Auswertung Fettsäuremuster **Anlage zur Proben-Nr.: 19FU045723, Eier & Eiprodukte**

Methode: ASU L 13.00-27/2; 2012-01 / ASU L 13.00-26; 2008-06 Oldenburg, 05.12.2019

gesättigte Fettsäuren (SFA)

Parameter	C-Zahl	%
Buttersäure	C4:0	n.b.
Capronsäure	C6:0	n.b.
Önanthsäure	C7:0	n.b.
Caprylsäure	C8:0	0,1
Pelargonsäure	C9:0	n.b.
Caprinsäure	C10:0	0,2
Undecansäure	C11:0	n.b.
Laurinsäure	C12:0	0,5
Tridecansäure	C13:0	n.b.
Myristinsäure	C14:0	0,3
Pentadecansäure	C15:0	0,1
Palmitinsäure	C16:0	24,5
Margarinsäure	C17:0	0,3
Stearinsäure	C18:0	8,1
Arachinsäure	C20:0	n.b.
Hareicosansäure	C21:0	n.b.
Behensäure	C22:0	n.b.
Tricosansäure	C23:0	n.b.
Lignocerinsäure	C24:0	n.b.
Hexacosansäure	C26:0	n.b.

Einfach ungesättigte Fettsäuren (MUFA)

Parameter	C-Zahl	Stellung	Bes.	%
Myristoleinsäure	C14:1	9c		n.b.
Pentadecensäure	C15:1	10c		n.b.
Palmitoleinsäure	C16:1	9c		2,8
Palmitelaidinsäure	C16:1	9t	trans	n.b.
Heptadecensäure	C17:1	9t		n.b.
Ölsäure	C18:1	9c	ω-9	38,2
Elaidinsäure	C18:1	9t	trans	0,2
Vaccensäure	C18:1	11c		1,7
	C18:1	11t	trans	0,2
Petroselinsäure	C18:1	6c		n.b.
Eicosensäure	C20:1	11c	ω-9	0,2
	C20:1	11t	trans	n.b.
Erucasäure	C22:1	13c	ω-9	n.b.
	C22:1	13t	trans	n.b.
Nervonsäure	C24:1	15c	ω-9	n.b.

mehrfach ungesättigte Fettsäuren (PUFA)

Parameter	C-Zahl	Stellung	Bes.	%
Linolsäure	C18:2	9c,12c	ω-6	13,9
	C18:2	9c,12t	trans	n.b.
	C18:2	9t,c12	trans	0,3
	C18:2	9t,12t	trans	n.b.
	C18:2	9c,t11	CLA	0,2
	C18:2	10t,c12	CLA	0,1
Eicosadiensäure	C20:2	11c,14c	ω-6	0,1
Docosadiensäure	C22:2	13c,16c	ω-6	n.b.
alpha Linolensäure	C18:3	9c,11c,15c	ω-3	5,6
gamma Linolensäure	C18:3	6c,9c,12c	ω-6	n.b.
Dihomo-gamma Linolensäure	C20:3	8c,11c,14c	ω-6	0,1
Arachidonsäure	C20:4	5c,8c,11c,14c	ω-6	0,7
Docosatetraensäure	C22:4	7c,10c,13c,16c	ω-6	n.b.
Eicosapentaensäure	C20:5	5c,8c,11c,14c,17c,	ω-3	0,1
Docosapentaensäure	C22:5	7c,10c,13c,16c,19c	ω-3	0,3
Docosahexaensäure	C22:6	4c,7c,10c,13c,16c,19c	ω-3	0,6

Parameter	%
Summe der gesättigten Fettsäuren	34,2
Summe der einfach ungesättigten Fettsäuren	43,2
Summe der mehrfach ungesättigten Fettsäuren	22,0
Summe der konjugierten Linolsäure-Isomere	0,3
Summe der trans-Fettsäuren	0,6
Summe der ω-3 Fettsäuren	6,6
Summe der ω-6 Fettsäuren	14,9
Summe der ω-9 Fettsäuren	38,4

% - Angabe bezieht sich auf den Fettsäureanteil
n.b. - nicht bestimmbar

Im Auftrag

Dr. Andrea Kuhr
Leitung Rückstands- und Radioaktivitätsuntersuchungen

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Figure C.25: Lab report of the FA profile of the chicken eggs of the experimental group (Experiment 6).

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Prüfbericht

Oldenburg, 05.12.2019
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Berichts-Version: 1

Kunden-Nr.:	50178422	Probeneingang:	05.11.2019
Auftrags-Nr.:	1850734	Untersuchungsbeginn:	05.11.2019
Proben-Nr.:	**19FU045723**	Untersuchungsende:	05.12.2019
Probenart:	Eier & Eiprodukte		
Befindlich in:	Pappkarton		
Bezeichnung:	V 01.11.2019		
Probenehmer:	durch Auftraggeber		
Probenzustand:	Probeneingang gekühlt		

	Ergebnis in der Originalsubstanz
Rohfett A+B *VO (EG) 152 Anhang III, H; 2009*	**9,5 %**
Vitamin A *LUFA Nord-West 1/3-029; 2017-11*	**7700 IE/kg**
Vitamin E, berechnet als alpha-Tocopherolacetat *LUFA Nord-West 1/3-029; 2017-11*	**40 mg/kg**
Fettsäuremuster *ASU L 13.00-26; 2008-06 / ASU L 13.00-27/2; 2012-01*	**siehe Anlage**

Im Auftrag

Dr. Michael Egert
Institutsleiter

Dieser Prüfbericht wurde maschinell erstellt und ist ohne Unterschrift gültig.

#2 = IfT, Oldenburg; #3 = IfL, Oldenburg; #4 = IfB, IfD, Hameln; #5 = Untersuchung erfolgte in Fremdlabor; #6 = unterliegt nicht der Akkreditierung
„<…" = Wert ist kleiner als die nebenstehende untere Grenze des Arbeitsbereiches

Die Untersuchungsergebnisse beziehen sich auf das uns vorliegende Probenmaterial. Dieser Prüfbericht darf nur vollständig und unverändert weiterverbreitet werden. Abweichende Vorgehensweisen bedürfen der schriftlichen Genehmigung der LUFA Nord-West. Die Akkreditierung gilt für den in der Urkundenanlage D-PL-14165-01-00 festgelegten Umfang.

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Figure C.26: Lab report of the nutritional analysis of the chicken eggs of the experimental group (Experiment 6).

Table C.1: Several parameters of the pond inside the polytunnel (P) and the outside pond (O).

	Temp. [°C]		pH		EC [µS/cm]		D. O. [mg/l]		NO3--N [mg/l]		NH4+-N [mg/l]		TP-P [mg/l]		TN [mg/l]		TOC [mg/l]		TIC [mg/l]		TC [mg/l]	
	O	P	O	P	O	P	O	P	O	P	O	P	O	P	O	P	O	P	O	P	O	P
26/03/2019	8.9	11.1	9.01	7.37	1348	1125	17.58	7.18	3.51	6.2	3.4	7.69	7.4	17.1	10.56±0.063	15.47±0.330	45.78±1.48	37.65±1.81	19.75±1.94	38.90±0.157	65,53 ± 0,457	76.56±1.97
02/04/2019	11.6	14.4	9.58	7.38	1337	1108	17.38	7.62														
09/04/2019	13.3	15.8	9.87	7.35	1298	1109	17.83	7.38	1.67	2.73	0.671	1.27	18.7	20.7	10.71±0.090	1023±0.047	71.15±0.321	39.80±1.35	17.47±0.170	32.03±0.125	88,63 ± 0,151	71.83±1.47
16/04/2019	13	16.6	9.81	7.25	1338	824	16.63	4.69														
23/04/2019	17	20.8	7.75	7.15	1503	1080	5.26	3.97	0.8	1.06	3.74	2.47	22.8	16.5	11.38±0.0046	549±0.166	59.60±0.082	33.64±0.898	35.05±0.287	35.00±0.632	94,65 ± 0,369	68.64±0.266
30/04/2019	17.5	20.5	7.3	7.17	1515	445	2.44	3.71														
07/05/2019	12	18.1	7.13	7.03	1440	1074	7.2	1.84	0.808	1.17	4.65	2.41	20.3	18.7	11.47±0.143	4.85±0.066	59.82±0.735	32.36±0.642	36.95±0.021	30.87±0.288	96,77 ± 0,714	63.22±0.931
14/05/2019	16.2	22	7.04	7.01	1448	1112	0.96	4.93														
21/05/2019	16.5	21.2	6.97	7.04	1467	1105	0.98	5.4	0.834	2.05	3.51	0.586	20.5	19.9	8.63±0.168	4.42±0.066	67.01±4.37	32.02±0.268	38.95±4.19	32.97±0.414	106,0 ± 0,188	64.99±0.682
28/05/2019	16.2	22	6.92	7.06	1492	1111	0.43	3.08														
04/06/2019	20.6	22.4	7.1	6.94	1479	1120	4.73	4.75	1.58	0.95	1.85	2.12	21.9	19	7.44±0.0070	2.38±0.064	52.10±0.476	32.28±0.582	39.55±0.138	29.75±0.143	91,65 ± 0,338	62.03±0.439
11/06/2019	18.1	23	6.93	6.97	907	1142	1.04	2.33														
18/06/2019	20.9	32.8	6.89	7.09	1068	1179	1.27	3.74	0.961	0.998	3.1	1.82	19.4	19.2	5.21±0.074	4.47±0.188	52.42±0.634	37.95±2.20	30.42±0.433	41.51±0.195	82,84 ± 0,201	79.46±2.00
25/06/2019	23.2	29.5	6.84	6.99	1024	947	3.07	1.48														
02/07/2019	20	24.2	7.12	7.08	1178	940	0.64	0.91	0.952	2.19	4.53	1.03	21.5	16.7	7.49±0.629	4.39±0.019	51.72±4.23	48.05±1.04	42.23±0.118	26.97±0.399	93,95 ± 4,11	75.02±1.44
09/07/2019	17.5	21.6	7.19	6.89	1130	892	3.16	1.43														
16/07/2019	17.9	23.3	6.88	6.75	1200	847	0.15	1.69	1.21	2.92	2.31	1.33	24.8	19	7.45±0.058	3.00±0.194	45.77±0.211	37.40±0.021	56.90±1.40	34.03±1.22	102,7 ± 1,19	71.43±1.20
23/07/2019	26.7	26.3	7.48	6.66	1240	866	1.99	0.33														
30/07/2019	22.2	25.6	7.08	6.68	960	839	1.54	2.86	0.939	1.13	3.87	0.282	23.8	19	6.36±0.013	3.16±0.022	39.53±1.19	37.02±0.591	60.11±0.573	40.10±0.917	99,64 ± 0,618	77.12±1.51
06/08/2019	24		7.84		1208		3															
13/08/2019	20.5		8.31		1324		4.35															

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