Development of a Goldfish Feed from Organic Sustainable Waste Sources

A report presented in partial fulfillment of the requirements of the degree of Bachelor of Technology (Honours) in Food Technology of Massey University

Adrian Hoggard

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Executive Summary

The feasibility and possibility of producing a goldfish feed from sustainable sources has been assessed. It was found that there is a high potential for a profit to be made on the product. The feed formulation contains duckweed, salad waste products, spirulina, seed waste products, flours, and vitamins and minerals. 61% of the formulation is deemed to be from sustainable sources with potential for it to be increased to 75%. Duckweed is a key ingredient because of its high protein content and favourable amino acid composition. It can also be grown on a waste stream pond by utilising the nutrients in it. The duckweed will need to be grown specifically for the fish feed as it is not currently available in New Zealand. It is essential to identify many ponds so that more duckweed can be grown, and more feed can be produced.

Extrusion was found to be the most likely method to process the feed. It can achieve the required physical properties whilst maintaining the required nutritional properties. It is believed that the goldfish feed will be suitable for goldfish to consume safely, but this will still need to be confirmed by doing an experiment to compare it to other foods on the market.

Further research will need to be done into packaging and marketing techniques for the product but initial indications suggest that there is potential for the product.

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Contents

Figures

Tables

1.0 Introduction

The purpose of Berrysmith Foundation is to provide and produce foods from sustainable sources, waste streams, or from foods which utilize waste streams. Sustainability of foods is of major concern with the world's population continuously increasing. It has been identified that there are no current goldfish foods in the market which are produced from sustainable sources. Most goldfish foods use fishmeal as a protein source, but there are questions around the sustainability of ocean fish, as 75% of fisheries are believed to be currently fished at, or above, the maximum level which can be maintained (FAO, n.d.). The purpose of the project was to produce a goldfish food from organic waste streams and other sustainable sources. The appropriateness of duckweed (*Lemna minor*) is also to be investigated because it can be growth on the nutrient waste run off from horticultural operations. Since the goldfish food will be aimed at the domestic market, it must be safe and nutritious for the fish. The presence of a sustainable goldfish feed in the market will be a good promoter for other sustainable foods.

1.1 Aim

To develop a sustainable goldfish food from industrial organic waste streams which is to be used as the major food source in the diet of the goldfish.

1.2 Objectives

- Literature Review:
    - Investigate current goldfish foods on the market (price, nutritional value, form, size).
    - Determine nutritional requirements of goldfish (amino acids, essential fatty acids, energy sources, vitamins and minerals).
    - Determine palatability of foods for goldfish, and ingredients which will make goldfish consume product.
    - Find possible food waste streams to use.
    - Find composition of sustainable ingredients which are available for product (protein, amino acids, lipid, essential fatty acids, water).
    - Determine optimal combination of ingredients to meet goldfish nutrient requirements, while minimising costs.
    - Calculate/determine optimal properties of goldfish food to ensure it is eaten before settling on bottom (density, water absorption rate)
    - Investigate possible methods of forming and drying product.
    - Investigate methods of testing acceptability and health/growth rates of goldfish.- Determine a safe water activity and methods to prevent/reduce unfavourable changes such as lipid oxidation.
- Experimental:
    - Combine ingredients and process using methods decided on in literature review.

    - Reformulate or try other methods if product is not acceptable.

- Product Testing:
    - Submit application to ethics committee describing testing process.
      Test the water activity of product.
    - Test the settling speed of the product in water.
    - Perform palatability and goldfish health/growth rate tests under the supervision of a fish vet.

1.3 Constraints

- Access to duckweed – Berrysmith Foundation and NZFC do not currently grow/process duckweed, and it is not commonly used in New Zealand.
- Ethical approval for testing product on fish – problems could arise if approval is not granted.
- Access to enough goldfish for testing.

2.0 Information summary

2.1 Current goldfish food products on the market

The current products on the market range in price from about $110.00/kg to $180.00/kg retail. The high-end products are often formulated to enhance the colour in the goldfish. It is likely that the price of the sustainable goldfish feed would need to be towards the lower end because it would not have the same properties as the expensive feeds. Also, people may perceive a non-meat fish food as being inferior, and may not want to purchase it for the price of the most expensive feeds.

The current goldfish products on the market contain a minimum level of protein between 30% and 48%. This goldfish feed should be formulated to contain a level the same as those on the market. Some fish foods have high proportions of spirulina (also known as blue-green algae or cyanobacteria) which helps to improve the protein content from non-meat sources. An 'American Aquarium Products' feed contains 20% spirulina (American aquarium products, n.d.). The lipid content is usually claimed to be greater than 3-6% and the fibre conent is claim to be no greater than 3-6% also. Ideally, similar claims should be able to be made, so these nutrient levels will be good guidelines for the goldfish feed.

2.2 Nutritional Requirements for Goldfish

2.1.1 Protein and Amino Acids

Goldfish (*Carassius* auratus) are part of the carp family. Adult carp require approximately 30% of their diet to consist of protein, while juvenile carp require closer to 40% (National Research Council, 2003). Jhingran and Pullin (1985) suggested that diets of 28-35% protein are optimal for adult carp. If excess protein is added to the diet, then it will be converted to energy. It is essential that the fish food is to contain "arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine" (National Research Council, 2003). Fish require these amino acids but cannot synthesise them themselves (Meyer, n.d.). Table 1 shows the essential amino acid requirements for carp.

Table 1: Essential amino acid requirements in a carp's diet.

Amino Acid	% of total protein	% of total feed (Assuming 30% protein)
Arginine	4.3	1.3
Histidine	2.1	0.6
Isoleucine	2.5	0.8
Leucine	3.3	1.0
Lysine	5.7	1.7
Methionine	3.1	0.9
Phenylalanine	6.5	2.0
Threonine	3.9	1.2
Tryptophan	0.8	0.2
Valine	3.6	1.1

Adapted from Table 1-4 of National Research Council (1993)

Generally, if the amino acid components are from free amino acids, then they are not absorbed as well as they are from proteins (National Research Council, 1993). Any amino acids should be added in the form of proteins. If the feed contains an adequate amount of protein and the correct amino acids, the protein source should be suitable in a goldfish food provided that it is digestible. Carp utilize plant proteins very well. They digest plant proteins only slightly less than proteins from meat sources (Webster and Lim, 2002).

2.1.2 Lipids and Fatty Acids

It is very difficult to assess the total amount of lipids needed by fish, because it depends on the lipids present and the total amount of energy provided by the food (National Research Council, 2003). Unlike many fishes, carp can utilize carbohydrates as an energy source, so the total energy content of the food is often considered instead of the individual carbohydrate and lipid content (Webster and Lim, 2002). If the food provides enough dietary energy, then the lipid levels can be ignored, provided that they are not excessive. Carp require two essential fatty acids in their diet. These are linolenic acid and linoleic acid and both need to be supplied as 1% of the goldfish's diet (National Research Council, 1993). Oxidated fatty acids can negatively influence carp in the same way as a vitamin E deficiency would. Extra vitamin E intake in the diet would counteract this (National Research Council, 1993).

2.1.3 Carbohydrates

Carp, and therefore goldfish, can use carbohydrates as an energy source. The use of starch results in a greater weight gain than other carbohydrates, but glucose and maltose are both utilised as well (Webster and Lim, 2002). Gelatinized starch, in particular, can be an effective energy source for carp (National Research Council, 1993). The optimal level of carbohydrate in a carp's diet is believed to be 30-40% (Webster and Lim, 2002).

2.1.4 Total Energy Requirements

The level of digestible energy in the fish feed is very important. Digestible energy is the total amount of energy that could be provided by the feed, excluding the energy content of the materials excreted in faeces. Digestible energy needs to be in balance with the dietary protein, because too much energy will result in a decreased intake of nutrients, but too little energy will result in protein being used as an energy source at the expense of muscle and tissue growth (National Research Council, 1993). In a diet containing 31.5% protein, carp need 2.90kcal of digestible energy per gram of feed.

2.1.5 Fibre

Fish require some fibre in their diets. Fibre makes food pass through the digestive tract and can increase the utilisation of proteins (National Research Council, 1993). Fibre should be present in levels not exceeding 8% to ensure that fish growth is not reduced (National Research Council, 1993).

2.1.6 Minerals

Calcium can be obtained by goldfish through absorption from the water, but some needs to be obtained through dietary sources. Calcium is required by the fish for bones and muscle contraction. It is recommended that a carp feed is to contain about 0.3% calcium for maximal growth (National Research Council, 1993).

Magnesium can be obtained through absorption from the water by the goldfish. It is estimated that carp require 0.04%-0.06% magnesium in their diet, but fish often obtain enough from their environment (National Research Council, 1993). Goldfish in a tank, without free flowing water, may require some magnesium in their diet since a reduced amount may be present in the water.

Iron is required in the carp's diet to prevent microcytic anaemia (small red blood cell formation). Some species of fish require 0.003%-0.02% iron in their diet, and some reach toxic levels once the iron exceeds 0.14% (National Research Council, 1993). It is estimated that 0.02% iron will provide carp with enough dietary iron without causing toxication.

Copper is required by carp in the diet at a level around 0.0003% (3mg/kg food) (National Research Council, 1993).

Zinc is important for fish as it is a component in many different enzymes. Dietary zinc can be quite important because it is absorbed more readily than that from the water. It is recommended that a feed is to contain approximately 0.003% (30mg/kg food) zinc (National Research Council, 1993).

Manganese is required by carp from the diet at a level around 0.0013% (13mg/kg food) (National research council, 1993). Some is absorbed from the water, but dietary manganese is absorbed more readily.

Phosphorous is needed by carp as it is used in the metabolism of proteins, fats, and carbohydrates. The level of phosphorous needed for goldfish is in the range of 0.5%-0.8%. Phosphorous bioavailability from plant sources is very low (about 30-50%) so any phosphorous present would have to be in a higher level than estimated if it originates from plant material (National Research Council, 1993). Phosphorous levels of 1.6% should be adequate. Monobasic calcium phosphate is the most bioavailable source for phosphorous fortification (National Research Council, 1993).

2.1.7 Vitamins

Many vitamins are needed by carp for a range of functions. Most are needed to prevent poor growth, anorexia, and skin degradation (Webster and Lim, 2002). The level of each vitamin required in the diet is shown in Table 2.

Table 2: Vitamin requirements for carp

Vitamin	Amount required (mg/kg)
Thiamin	0.5
Riboflavin	7
Pyridoxine	6
Panthothenic acid	30
Niacin	28
Biotin	1
Choline	500
Inositol	440
Vitamin A	4000 IU/kg
Vitamin E	0.01
Vitamin C	unkown, but needed

Adapted from Table 18.4 of Webster and Lim (2002)

The feed formulation should be aimed to contain an excess of the amount stated in table 2 because some vitamins may be degraded during processing (National Research Council, 1993). The expected vitamin losses during extrusion are shown in Table 3 (Shapleski, 2003).

Table 3: Expected vitamin losses during extrusion

Vitamin	Expected Loss (%)
Thiamin	6-62
Riboflavin	0-40
Pyridoxine	4-44
Niacin	0-40
Vitamin C	0-87
Fat soluble	<20

Adapted from table 7.1 of Shapleski (2003)

2.1.8 Possible antinutritional components

Raw soybeans should be avoided because they contain a trypsin inhibitor which can be devastating for fish (National Research Council, 1993). Trypsin is required for protein digestion (Schwartz, 2008). If soybeans are to be used in a fish feed, they must be heated to destroy the trypsin inhibitor but not degrade any essential amino acids.

Cottonseed meal could have negative effects on carp because of gossypol and cyclopropenoic fatty acids (CFA's) present. Gossypol can decrease growth and cause damage to organs in some species of fish (National Research Council, 1993). CFA's have been proven to cause "lesions, increased glycogen deposition, and elevated saturated fatty acid concentration in the liver in rainbow trout" (National Research Council, 1993). The effects of CFAs on carp is unknown, so cottonseed meal should be avoided if possible.

Oilseeds, such as rapeseed and sunflower, can contain glucosinolates which get hydrolysed to compounds which reduce iodine absorption (National Research Council, 1993). Extra dietary iodine can counteract these effects. Glucosinolates can also reduce growth rates in carp (National Research Council, 1993).

2.2 Palatability of feeds

Getting a fish to actually consume the food product may be difficult. The food will be taken into the mouth based on sight but it will only be swallowed if the taste is favourable (National Research Council, 1993). Therefore, it is very important to make the taste of the food appealing for goldfish. Carnivorous fish prefer alkaline and neutral food components but herbivorous fish prefer acidic. Carp are omnivorous but studies have shown that they exhibit a greater response to neutral and acidic substances than alkaline (Billard and Marcel, 1986). It is recommended, if possible, to use proteins with a high acidic amino acid composition as opposed to those with high alkaline amino acid compositions (National Research Council, 1993). High concentrations of aspartic acid and glutamic acid should help to promote the ingestion of the feed.

Some other compounds can also be used as feeding stimulants. The presence of dimethyl-β-propiothetin (also known as Dimethylsulfoniopropionate) in the food is known to attract carp to it (Nakajima *et al*, 1989). Algae and halophytic plants are good sources of dimethylsulfoniopropionate (Yoch, 2002). These could be incorporated into the feed to promote consumption.

2.3 Possible ingredients and their nutritional value

2.3.1 Possible components

Berrysmith Foundation has identified snow pea shoots, lettuce, and carrots as components which would be inexpensive and available to use if they can be incorporated into the feed. Duckweed (*Lemna* or *Spirodela*) and microalgae have also been identified as possible components which could be incorporated into the feed because they fit the Berrysmith Foundation image. Flaxseed waste and hempseed waste have also been identified as possible components due to their availability as a waste source and their essential fatty acid composition. The components all have to meet the nutritional requirements for the goldfish, as well as be able to be processed effectively.

2.3.2 Snow pea waste

New Zealand Fresh Cuts, who will supply most of the vegetable waste streams, have had their snow pea waste stream analysed for moisture and protein content. The moisture content was determined to be 78.7% and the protein content was 4.3% (20.2% of the dry matter of the snow pea waste). The other 17% would be carbohydrates, lipids, fibre, and ash. The protein proportion of the dry matter would be 20.2%. Foodworks suggests that the protein content is 3.99%, fat content is 0.23% fibre content is 0.8% and the moisture content was 93% of the total matter. However, Foodworks does not give any information about the carbohydrate content and the total content does not total 100%. This data has been used for the formulation since other data is limited.

A sample of snow pea waste was received from New Zealand Fresh Cuts and it was washed and a proximate analysis was performed on it to determine the true macronutrient composition. The air oven method was used for moisture determination, soxhlet extraction for fat determination, Kjeldahl method for protein determination, and muffle furnace for ash determination, as outlined in Food Chemistry (2008). It was found to be 81.5% moisture. The dry matter of it was found to contain 19.1% protein, 1.3% lipids, 2.9% ash, and an estimated 50% carbohydrate and 29.6% fibre. The calculated values are consistent with the data supplied by New Zealand Fresh Cuts.The proximate analysis raw data and calculations are presented in appendix 12.2.

2.3.3 Carrot waste

The composition of the carrot waste is shown in appendix 12.1.1. The total protein content is very low (about 7%) so it is unlikely that much carrot could be incorporated into the product (National food institute). Problems could also arise with carrots because they may need to be softened first, and this may involve cooking which could denature some of the nutrients. The main carbohydrate content of carrots are glucose, fructose and saccharose (National food institute) which are not the most digestible carbohydrates for carp. Carrots have a high linoleic acid (National food institute) content which is required by carp and could be useful to incorporate into the feed.

2.3.4 Lettuce waste

The composition of lettuce is shown in appendix 12.1.2. It contains 95.1% moisture (National food institute). 28.57% of the dry matter of lettuce is protein (National food institute). This is almost the required level of protein for carp. However, lettuce would not be appropriate as a major component of the goldfish feed because it has a very high fibre content (26.53% of the dry matter) (National food institute). Lettuce is a reasonable source of linolenic acid (National food institute), so its inclusion in a feed could be beneficial. It will also provide vitamin C, which is needed by carp.

2.3.5 Duckweed

The composition of duckweed is shown in appendix 12.1.3. Duckweed has a very high protein content for a plant. The protein content is about 41.7% of the dry matter of duckweed which has been grown in nutrient rich conditions. The moisture content in duckweed is approximately 93% (Landesman *et al*, n.d.). The essential amino acid profile of duckweed is quite similar to that needed by carp. This means that the protein content of duckweed is almost perfect because it is in a high concentration and it also provides most of the essential amino acids. Unfortunately, the essential fatty acid, vitamin, and mineral content is unknown so if it incorporated, other foods would have to make up these requirements to ensure that it is suitable for the goldfish.

Duckweed is not commonly grown and harvested in New Zealand, and therefore is not easily available. If the correct nutrients are available, duckweed can grow up to 183 MT/ha/yr (approximately 700g/m^2/day). The best conditions for growing duckweed include; slow flowing water, 6°C-33°C, and the presence of decaying organic material (Skillicorn *et al*, 1993).Since duckweed grows so quickly and readily, a problem may arise with duckweed growing from the feed after it has been produced. The unwanted growth of duckweed in a fish tank, if there is any, could be assessed when the feed is tested on the goldfish

2.3.6 Spirulina

The composition of spirulina is shown in appendix 12.1.4. Spirulina has a very high protein content. It composes about 63% of the dry matter (Life research universal, 2004). Spirulina has an incredibly high Vitamin A content, which could be toxic if the spirulina is included in a too large proportion (New life international, 2009). New life international (2009) mention that 5-10% spirulina inclusion in a fish feed will increase growth rates. It is estimated that the spirulina content is to not exceed 10%. Spirulina has a very high iron content (about 0.16% of the dry matter) which will be in low concentrations in most of the other possible ingredients.

2.3.7 Pea flour

The composition of pea flour is shown in appendix 12.1.5. Pea flour could be very important to the feed because it contains a high starch content, which is required for extrusion. The starch can also provide energy for the goldfish (Webster and Lim, 2002). Pea flour would be a good high-starch product to include because it also contains a reasonable level of protein. It provides linoleic acid (National food institute), which is an essential fatty acid for carp (National research council, 1993).

2.3.8 Corn flour

The composition of corn flour is shown in appendix 12.1.6. It has a very high starch content, but low protein content (National food institute). The starch is needed for extrusion, but the low protein content is bad since the total protein content of the feed needs to be about 30%. The low fibre content of the corn flour is very beneficial because the fibre content of the feed is recommended to not exceed 8%.

2.3.9 Flaxseed and hempseed cake

The flaxseed cake and hempseed cake could both be useful due to the essential fatty acid composition of them. The flaxseed cake has a high linolenic acid composition, and the hempseed cake has a high linoleic acid composition (A. Davidson, personal communication, May 15, 2009).

2.4 Physical properties required for the feed

2.4.1 Ideal properties

Carp generally feed on food which is descending (P. Davie, personal communication, March 27, 2009). Ideally, the food will not sink too quickly in a fish tank because once it has hit the bottom, it is generally not consumed. It is not such a problem in aquaculture situations, where the distance to the bottom is far. Sedimentation of feeds can promote the growth of algae and also increase the nitrogen level in the water (A. Hardacre, personal communication, May 8, 2009), which are both unfavourable.

Ideally the feed will:

- Have consistently sized particles of about 2mm in diameter.
- Initially have a particle density of less than 1000kg/m^3 so it floats.
- Absorb water slowly so it will start to sink slowly.
- Be water soluble for easy consumption by the fish (National research council, 1993).
- Not be too powdery (Guillaume *et al*, 2001).
- Not be too doughy or too hard (Guillaume *et al*, 2001).

2.4.2 Possible processing methods to achieve required physical properties

2.4.2.1 Pelleting

National research council (1993) suggests two ways of processing feeds for fish. The first is compression pelleting. High pressure, and usually high temperature, is required. The high temperature gelatinizes the starch, which helps to bind the feed (National research council, 1993). The high pressure, which is often caused by steam, compresses the feed into the dense pellets (National research council, 1993). Fibre reduces the binding ability of the pellets (National research council, 1993) so, for this formulation, pelleting is not ideal since most of the possible key ingredients have a high fibre content. Also, pelleting is not a good option because it produces very dense particules which will sink quickly and not be consumed. Due to both of these reasons, pelleting

2.4.2.2 Extrusion

The second method involves extrusion. National research council (1993) mentions that extrusion is done at high temperature and pressure. This would be unfavourable because the high temperature could destroy many of the nutrients in the feed. It is possible to extrude at a lower temperature and pressure (A. Hardacre, personal communication, May 8, 2009) so this could be done instead. Since high temperatures are not being used, the starch content of the pea flour will not gelatinize. This will result in poor binding of the materials and lower digestibility for the carp. Better binding increases the water stability of the feed (National research council, 1993) and, therefore, the time which the fish has to consume the feed before it disintegrates. The pea flour may have to be heated before being combined with any other ingredients to ensure that it gelatinizes. The raw materials would need to be dried substantially beforehand so that the total moisture content is about 25% (National research council, 1993). They would then be ground up and combined to make a paste/dough. When the mixture is fed through the extruder, and forced out the die holes, the water vapourises and forms gas bubbles in the mixture (National research council, 1993). The product would then need to be dried to a moisture content of approximately 5% and cut into the correct sized pieces.

Extrusion seems to be able to meet all of the criteria for the goldfish feed. The density of the particles would be able to be adjusted by changing the processing conditions in the extruder. A decreased moisture content or decreased pressure difference across the die holes of the extruder will result in a greater density of the product. Due to this, extrusion will be used for processing the goldfish feed.

3.0 Growth of Duckweed

3.1 Introduction

Duckweed is a key component of the goldfish feed because it has a very high protein content for a plant. It is not commonly grown in New Zealand so the availability of it is poor. For the purpose of the project, duckweed (*Lemna minor)* was grown in a glasshouse at the Plant Growth Unit (PGU) of Massey University, Palmerston North. It was grown there for two reasons; because it could effectively simulate the future growing conditions of the duckweed whilst monitoring them, and because input from the PGU employees was essential.

The objectives of growing the duckweed were:

- To grow enough duckweed to complete all of the required processing trial runs.
- To simulate the conditions in which the sponsor will grow his own duckweed, and determine the optimal harvesting rate.

3.2 Materials and Methods

3.2.1 Tank set-up

A glasshouse at the PGU was used because the temperature could be controlled and monitored to keep it similar to the outdoor temperature in Auckland. A thirty six tank setup was used, which comprised of six rows of tanks with six tanks in each row (as shown in figure 1). The total surface area of all of the tanks was 7m^2.

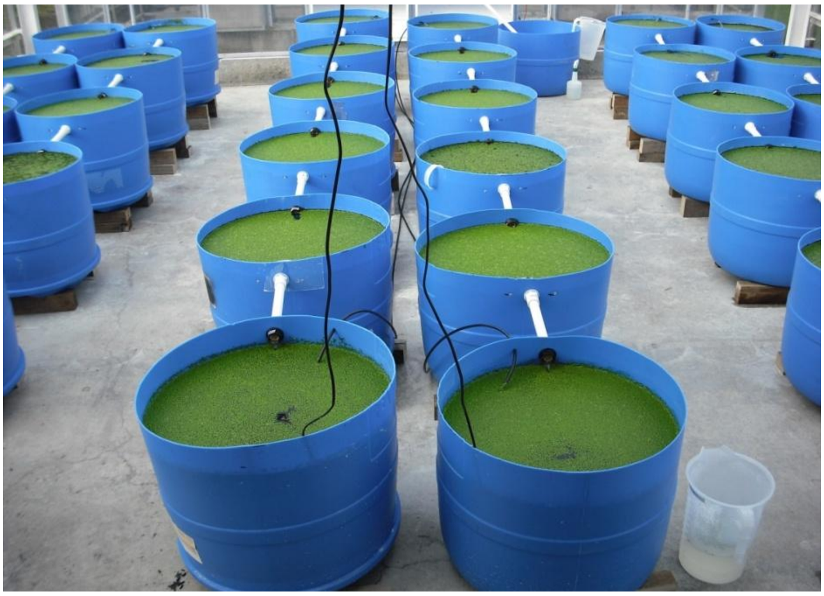

Figure 1: Tanks of duckweed in a glasshouse at the Plant Growth Unit.

Each tank was half of a 44 gallon drum, and were connected together using PVC piping. A barrier was attached in front of the piping to prevent the duckweed from being sucked through into the lower tank. The tanks were at different heights so that gravity would allow the water to drain from the top to the bottom, and the water would be pumped from the bottom tank back to the top to recirculate it. This allowed some flow of water to simulate the expected water movement in a large pond. The tanks were filled with a fertiliser solution with a nutrient composition (Appendix 12.3) which was similar to that of the horticultural waste run-off which the duckweed is expected to be grown in. The nutrient levels and pH were monitored using a nutrient meter and pH meter, and were adjusted to keep them constant throughout the entire experiment. Having six tanks in each row reduced the effect of any fluctuations in nutrient conditions or pH because it was spread over more tanks.

3.2.2 Collecting, growing, and harvesting duckweed

Wild duckweed (*Lemna minor*) was collected with a bucket and a small amount was transferred into each tank. The duckweed was left to grow until it formed a complete, thick cover. There was no actual measurement taken, but, instead, a judgement was made that the duckweed had grown enough to begin harvesting. The pictures in figure 4 show the growth of the duckweed. Once the duckweed in all of the tanks had reached the density shown in the right hand picture, the decision was made to begin harvesting.

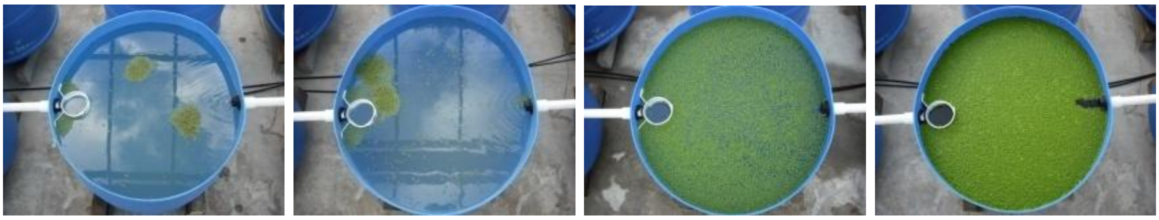

Figure 2: Duckweed growth - weeks 1 to 4.

Duckweed was scooped out of each tank by hand and put into a bucket in 2-3 day intervals. The amount of duckweed that was harvested each day was based on a visual judgement of the harvesting rate which could be sustained. It was collected from the densest patch in each tank. The duckweed, which had been harvested, was later dried.

3.3 Results and Discussion

The harvesting rate, for the conditions which the duckweed was grown in, was found to be 0.6215kg/day. Figure 3 is a plot of the total mass of duckweed harvested over time.

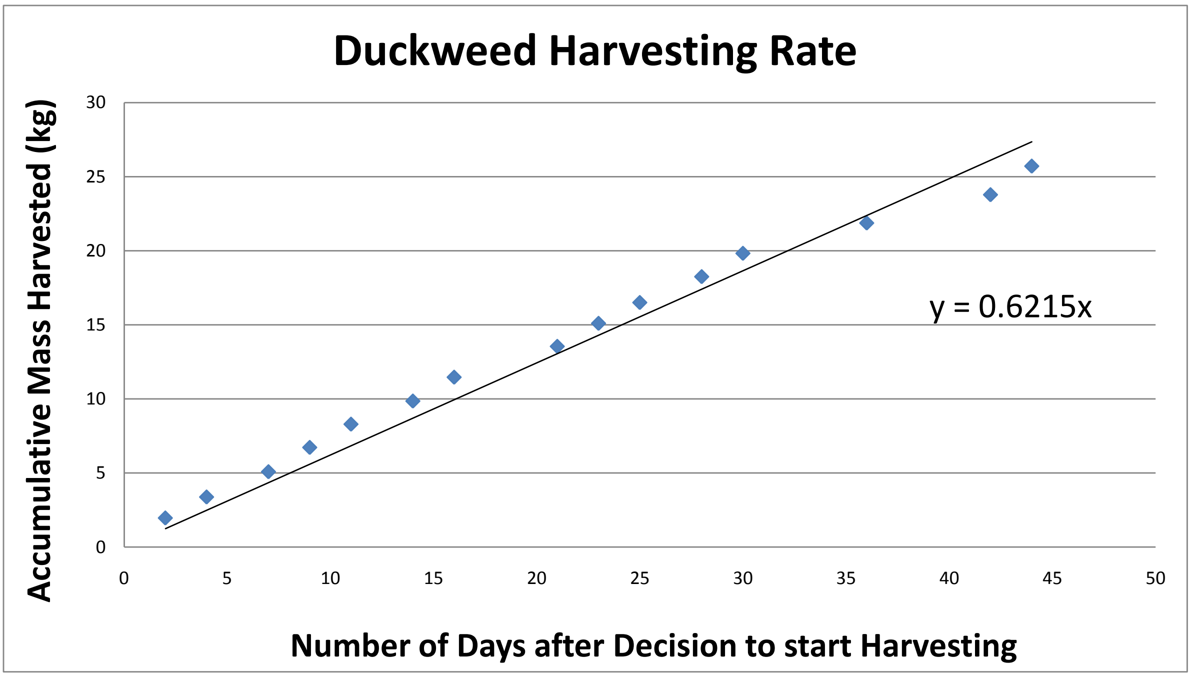

Figure 3: Plot of the total mass of duckweed harvested over time.

Given that the total surface area of the tanks was 7m^2, it has been calculated that duckweed can be harvested at a rate of 89g/m^2/day. The experimental value is consistent with that of literature. Skillicorn *et al* (1993) suggested that duckweed should be harvested at a rate of 100g/m^2/day to maximise the amount harvested, and to maintain the healthiest crop. The pond which the duckweed is going to be grown in is approximately 400m^2, so it is calculated that 35.5kg of fresh duckweed could be harvested per day. This value severely limits the amount of fish food that could be produced, so a larger area will need to be identified.

The key factors which result in optimal duckweed growth are; slow flowing water, warm temperatures, sunlight exposure, regular harvesting with a thick coverage, and continuous nutrient supply (Skillicorn *et al*, 1993). The flow rate of the water was slow in the trial, which optimises growth and is also similar to the expected duckweed growth conditions. The temperature of the glasshouse was maintained at a minimum of 17°C during the nights, and a maximum of 19°C-25°C during the days (Figure 4). Obviously, the maximum day temperature inside the glasshouse was colder during the winter months and increased as the growth trial progressed into spring.

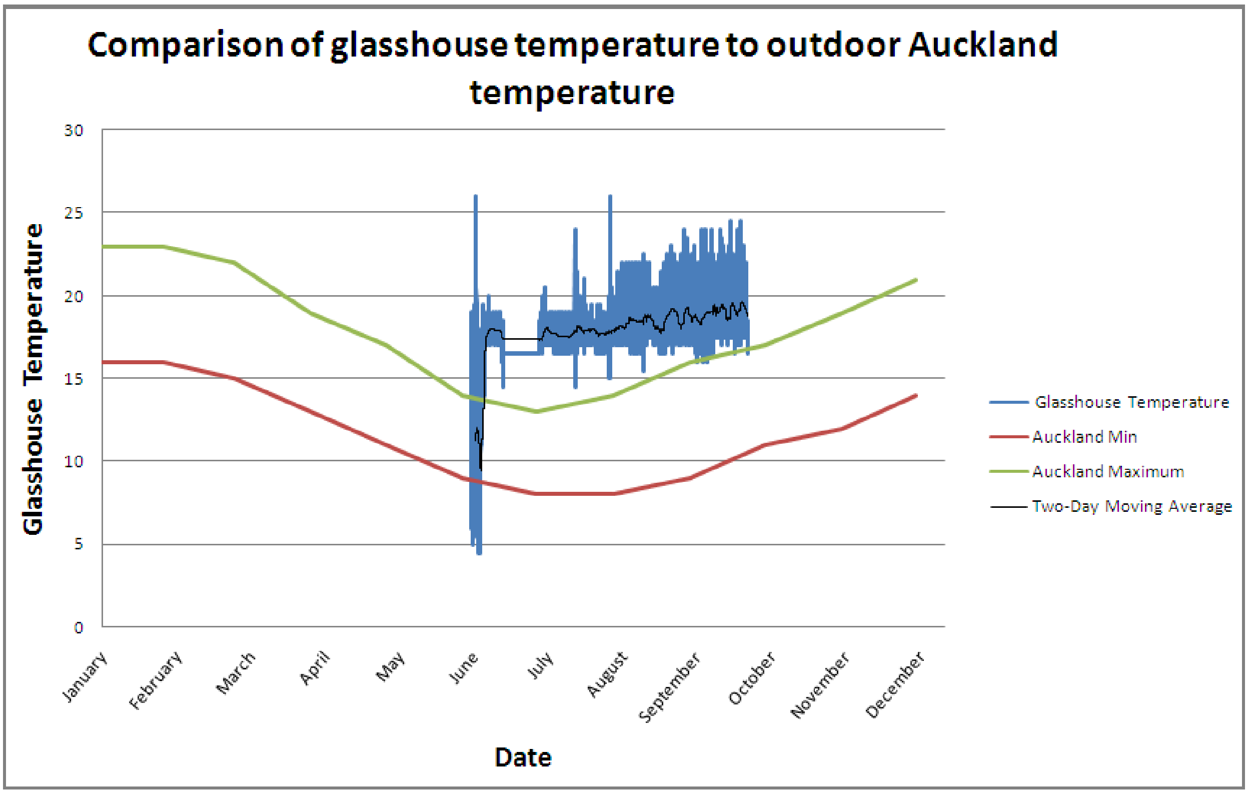

Figure 4: Comparison of growth temperature during trial to actual expected temperature.

It can be seen that the temperature in the glass house was in excess of the outdoor temperature in Auckland during the trial period. The average glasshouse temperature was more comparable to the average outdoor temperature in Auckland for the summer months. Although the growth trial temperature simulated an Auckland summer rather than an Auckland winter, it cannot be assumed that the calculated growth rate is indicative of a summer growth rate. During summer, the duckweed would be exposed to more sunlight and of a greater intensity, which would increase the duckweed growth rate (Skillicorn *et al*, 1993). Therefore, it is likely that during summer the duckweed harvesting rate will be greater than the experimental rate of 89g/m^2/day. The winter growth rate is likely to be less than the calculated experimental rate because of the reduced temperatures involved. Figure 3 suggests that the duckweed growth rate decreased towards the end of the trial, which coincided with the glasshouse temperature increasing. This would not be due to the increase in temperature. Instead, it would be due to the decrease in regularity of harvesting.

A problem arose during the growth of the duckweed because algae grew and seemed to suppress the growth of the duckweed (as shown in figure 5). Skillicorn *et al* (1993) said that algae should not grow in the presence of duckweed, because a thick layer of duckweed will block out light which suppresses the algal growth. Obviously this was not the case during the experiment, and it is unknown why the algae did grow when there was already a thick duckweed layer.

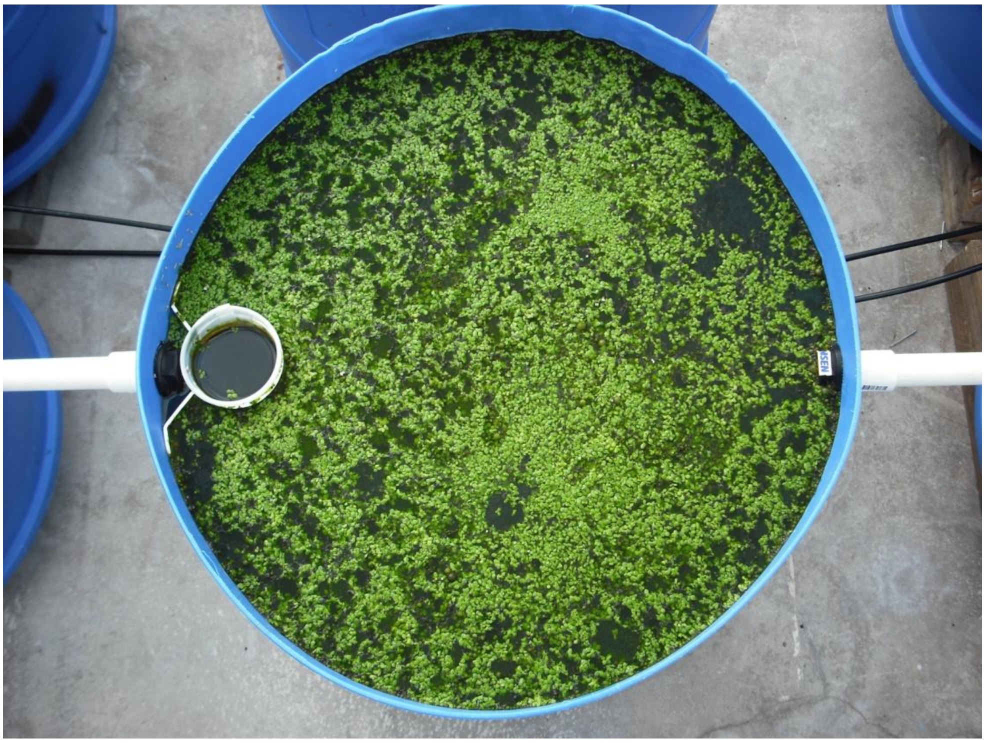
Figure 5: Tank which has been affected by algae

The solution to the algae problem was to dose the water with fish tank algaecide it regular intervals. Appendix 12.4 is a log of everything that was done to the tanks during the experiment. Approximately twice per week the tanks had algaecide mixed into at the recommended concentration on the bottle, or the duckweed/algae were directly sprayed with the algaecide solution (diluted to the concentration as recommended on the bottle). The source of the algae could not be identified because it is unknown if it was introduced with the wild duckweed, or if it was introduced afterwards. The sudden growth of algae coincided with the switch from chlorinated water to dechlorinated water. It suggests that the traces of chlorine that were being added during top-ups could have been suppressing the algal growth. Unfortunately, chlorine is unlikely to be present in the pond which the duckweed will be grown in, so algaecide may have to be used if algae does grow. Seeing as there are no current algae issues in the pond, there is also the possibility that algae will not grow when the duckweed is introduced. The current nutrient waste run-off may already have algaecide added to it, but this is unknown. The use of algaecide is expected to pose no health risks to goldfish that are fed the duckweed because the duckweed is washed, and the concentration of the algaecide in the food will be much less than the concentration that the algaecide is recommended to be used in fish tanks anyway.

The duckweed is expected to grow efficiently in the horticultural waste run-off ponds, but it is not expected to utilise a significant amount of the nutrients. One of the purposes of growing duckweed on the nutrient run-off ponds, was to help denitrify them, but this is not expected to happen because the rate which the duckweed uses the nutrients is minimal. This was shown in the experimental work because it was rare to need to top the tanks up with additional nutrients to maintain the conductivity factor (cf) value of 25. The cf value is a measure of nutrient concentrations in the water. Instead, the tanks were just topped up with water to compensate for the losses through evaporation.

3.4 Conclusions

- The average expected duckweed harvest rate is calculated as 89g/m^2/day.
- Warmth, light, slow-flowing water, continuous nutrient availability, thick coverage, and regular harvesting are the key factors to maximise harvest yield and to maintain a healthy crop.
- Harvesting is likely to show seasonality, with more being harvested in summer, and less in winter.
- Algae growth is inhibitory to duckweed growth, but the algae can be suppressed with fish tank algaecide diluted to the concentration mentioned on the bottle. With the current nutrient run-off pond, it is not expected that algae will grow.
- Approximately 35.5kg of fresh duckweed could be harvested from a 400m^2 pond, which limits the amount of goldfish food that could be produced.

4.0 Ingredient selection

The ingredients that were used were those which had been identified in the initial literature review. The carrot waste was found to have a poor nutritional composition for goldfish so it was eliminated as an ingredient for the goldfish food. The other key ingredients that had been identified in the initial literature review all provided crucial nutrients so were all included in various levels. The proportions of the dry matter of each ingredient which is required to meet the nutritional needs of goldfish is presented in table 4. The rows express to amount of each nutrient as a percentage of the total solids for each ingredient. The 'TOTAL COMPOSITION (%)' row expresses the amount of each nutrient as a percentage of the total solids of the entire feed. This value is then compared to the nutritional requirements and says 'yes' if it meets them, and 'no' if it doesn't.

NOTE: This table is available under the 'Formulation' tab in the excel spreadsheet which is named 'Formulation.xls'.

Table 4: Proportions of each ingredient used in the goldfish feed.

		Components (Dry matter %)				Essential Amino Acids (Dry matter %)									
	Dry matter %	Protein	Lipid	Carbohydrate	Fibre	Arginine	Histidine	Isoleucine	Leucine	Lysine	Methionine	Phenylalanine	Threonine	Tryptophan	Valine
Snow Pea Waste	7	19.14	1.3	50	29.56										
Carrot	0	6.93	3.96	58.42	28.71	0.218	0.0396	0.198	0.257	0.238	0.059	0.178	0.178	0.059	0.297
Lettuce	16	28.57	4.08	30.61	26.53	1.469	0.449	1.735	1.592	1.735	0.327	1.122	1.204	0.184	1.429
Duckweed	27	41.7	4.4	17.6	15.6	2.14	0.73	1.66	2.89	1.85	0.64	1.75	1.68	0.40	2.12
Spirulina	12	63.16	5.26	15.79	8.42	4.389	1.021	3.579	5.516	2.958	1.432	2.863	3.263	0.916	4.084
Pea flour	10.8	24.31	2.32	70.61	8.18	2.210	0.541	1.017	1.547	1.657	0.188	1.017	0.818	0.199	1.326
Corn Flour	14	7.65	3.15	84.93	3.60	0.315	0.214	0.281	0.967	0.214	0.146	0.394	0.281	0.049	0.371
Hominey	0														
Riboflavin Universal*	0.0007														
Calcium D-pantothenate*	0.006														
Dry Vitamin E 50% CWS/S*	0.08														
D-Biotin*	0.0003														
Ascorbic Acid*	0.1														
ROCOAT® Niacinamide 33⅓%*	0.002														
Pyridoxine Hydrochloride*	0.001														
Methionine	0	100									100				
Phenylalanine	0	100										100			
Lysine	0	100								100					
Monobasic Calcium Phosphate	1.5														
Zinc Sulphate	0.01														
Hemp Seed Cake	6	30.10	13.70	25.60	24.80	2.917	0.942	1.119	1.657	0.922	0.378	1.172	0.771	0.295	1.481
Flax Seed Cake	5.5	37.55	17.39	12.74	27.34	4.046	1.093	1.750	2.406	1.640	0.765	1.968	1.422	0.514	2.078
TOTAL COMPOSITION (%)	100	32.32	5.03	36.80	15.92	2.02	0.60	1.47	2.23	1.49	0.50	1.34	1.29	0.32	1.69
	FEED REQUIREMENTS	30-40	3-8	30-40	8 max	1.29	0.63	0.75	0.99	1.71	0.93	1.95	1.17	0.24	1.08
	EXCEEDS MINIMUM	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	No	No	No	Yes	Yes	Yes
	UNDER MAXIMUM	Yes	Yes	Yes	No										

		Essential fatty acids (Dry matter %)		Minerals (Dry matter %)						
	Dry matter %	Linolenic	Linoleic	Calcium	Magnesium	Iron	Copper	Zinc	Mangenese	Phosphorous
Snow Pea Waste	7			0.0951	0.2143	0.0101	0.0017	0.0089	0.0034	1.0043
Carrot	0	0.287	2.040	0.2436	0.0950	0.0024	0.0004	0.0019	0.0036	0.3277
Lettuce	16	1.143	0.469	0.7347	0.2653	0.0176	0.0006	0.0037	0.0051	0.5918
Duckweed	27									
Spirulina	12	1.053	0.658	0.7368	0.4211	0.1579	0.0013	0.0032	0.0053	0.8421
Pea flour	10.8	0.218	1.048	0.0418	0.0906	0.0061	0.0008	0.0042	0.0013	0.4497
Corn Flour	14	0.045	1.282	0.0067	0.0529	0.0012		0.0006	0.0000	0.1114
Hominey	0									
Riboflavin Universal*	0.0007									
Calcium D-pantothenate*	0.006			8.4						
Dry Vitamin E 50% CWS/S*	0.08									
D-Biotin*	0.0003									
Ascorbic Acid*	0.1									
ROCOAT® Niacinamide 33⅓%*	0.002									
Pyridoxine Hydrochloride*	0.001									
Methionine	0									
Phenylalanine	0									
Lysine	0									
Monobasic Calcium Phosphate	1.5			17.1200						26.4700
Zinc Sulphate	0.01							40.5000		
Hemp Seed Cake	6	3.315	7.384	0.2300	0.4900	0.0195				1.0900
Flax Seed Cake	5.5	9.563	2.782	0.6000						
TOTAL COMPOSITION (%)	100	1.06	1.04	0.52	0.15	0.0245	0.0004	0.0062	0.0018	0.79
	FEED REQUIREMENTS	1.00	1.00	0.3	0.04	0.003	0.0003	0.003	0.0013	0.8
	EXCEEDS MINIMUM	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
	UNDER MAXIMUM									

		Vitamins (Dry matter %)											
	Dry matter %	Thiamine	Riboflavin	Pyridoxine	Panthothenic acid	Niacin	Biotin	Choline	Inositol	Vitamin A (IU/kg)	Vitamin E	Vitamin C	Starch
Snow Pea Waste	7	0.0026	0.0020			0.0171				0.0386	0.0000	0.0000	
Carrot	0	0.0004	0.0003	0.0012	0.0028	0.0099		0.0871		0.0969	0.0054	0.0694	
Lettuce	16	0.0014	0.0016	0.0018	0.0027	0.0107		0.2735		0.0233	0.0000	0.3673	
Duckweed	27												
Spirulina	12	0.0037	0.0042	0.0008	0.0001	0.0147	0.0000		0.0674	0.1474	0.0105	0.0000	
Pea flour	10.8	0.0009	0.0002	0.0001	0.0002	0.0061	0.0000	0.0000	0.0000	0.0014	0.0000	0.0011	43.54
Corn Flour	14	0.0004	0.0001	0.0004		0.0006				0.0001	0.0013		83.46
Hominey	0												
Riboflavin Universal*	0.0007		99.5										
Calcium D-pantothenate*	0.006				91.1								
Dry Vitamin E 50% CWS/S*	0.08										33.5		
D-Biotin*	0.0003						99.25						
Ascorbic Acid*	0.1											99.75	
ROCOAT® Niacinamide 33⅓%*	0.002					32.6							
Pyridoxine Hydrochloride*	0.001			99.75									
Methionine	0												
Phenylalanine	0												
Lysine	0												
Monobasic Calcium Phosphate	1.5												
Zinc Sulphate	0.01												
Hemp Seed Cake	6												
Flax Seed Cake	5.5												
TOTAL COMPOSITION (%)	100	0.0000	0.0000	0.0000	0.006	0.0000	0.0003	0.0438	0.0081	0.0000	0.000	0.0000	16.39
	FEED REQUIREMENTS	0.00005	0.0007	0.0006	0.003	0.0028	0.0001	0.05	0.044	0.0003	0.01		15-20
	EXCEEDS MINIMUM	No	No	No	Yes	No	Yes	No	No	No	No	No	Yes
	UNDER MAXIMUM												Yes

* Mass added accounts for losses during processing

4.1 Sustainable content

The sustainable materials (snow pea waste, carrot waste, lettuce waste, duckweed, flax seed cake, and hemp seed cake) contribute to 61.5% of the total mass of dry solids in the feed. Other non-sustainable sources have been incorporated because the nutritional requirements for the feed could not be met without them. The spirulina is essential because it has a high protein content which is otherwise uncharacteristic of plant materials, and it also provides many vitamins which are essential. The pea flour and corn flour are required for their starch content. The starch is required for binding during extrusion so the particles can be held together. There may be a possibility to use a high-starch co-product from the flour milling process instead, such as hominey, but it is often already being fully utilised so it may not be considered as sustainable. Corson Grain produce hominey but it is sold at a price which is almost that of corn flour because there is a demand for it from the stock-feed and pet food industries (H. Cheetham, personal communication, September 15, 2009). Hominey was not used because the price is similar to that of corn flour and, nutritionally, it is worse because it has a greater fibre content (National research council, 1993). There is the possibility of directly substituting hominey for corn flour and it is expected, but not known, that this will have minimal influence over the ability to process to feed. A sustainable source of hominey will need to be identified. The advantages of making that substitution will be that the product could be deemed as having a larger 'sustainable' content, and that it will reduce some costs. The key disadvantage is that it will increase the fibre content, which may decrease growth rates in fish (National research council, 1993).

4.2 Macronutrient content

The macronutrient content has been optimised using the snow pea waste, carrot waste, lettuce waste, duckweed, spirulina, pea flour, corn flour, and hemp and flax seed cake. The protein, lipids, and carbohydrate levels would all be suitable for goldfish. However, the fibre level is in excess of the 8% maximum recommendation. This is unavoidable with the constraint that animal materials cannot be used. The high fibre content may result in reduced growth rates of the goldfish, but this could be determined when the feed is tested on the fish. If the goldfish do exhibit slower growth rates than those on a control diet, then it is probably attributable to the high fibre content. If this is the case, then further research will need to be conducted into removing fibre from the plant material. The use of enzymes could be a possible solution, but it will add additional costs to the process.

4.3 Micronutrient content

Most of the micronutrients are in excess of the recommended requirements in a goldfish's diet. Lysine, methionine, and phenylalanine are essential amino acids which are not in levels which meet the recommendations. Free amino acids are not absorbed as readily as amino acids from a protein source (National research council, 1993). Due to this, no free amino acids have been added to the formulation. The goldfish will also obtain amino acids from other parts of their diet, such as algae in their tank (P.Davie, personal communication, March 27, 2009). The algal component in the feed (spirulina) was the best source of the three required amino acids, so it is expected that the algae in the tank will contain them. If it is found that the growth rates of the test fish are less than that of goldfish on a control diet, then it may be worthwhile to incorporate them. It must be noted that the availability of food grade free amino acids in New Zealand is low, as many of the major nutrient suppliers do not have them. It is probable that the addition of them will add unnecessary and large costs to the formulation.

The vitamins which were required to be added are; riboflavin, panthothenic acid, vitamin E, biotin, ascorbic acid, niacin, pyridoxine, choline, and inositol. Choline and inositol were not available from Invita NZ Limited, who supplied the other vitamins that were required. This is not believed to be a problem, because the choline and inositol content of most of the ingredients was unknown, so the calculated content in the whole feed is lower than it should be, and goldfish obtain many of their nutrients from algae which are present in the tank already (P.Davie, personal communication, March 27, 2009). Spirulina is also an algae, and it contains high levels of inositol, so it is expected that algae in the tank will do so also. The other vitamins that were lacking have been incorporated through dry powders. The amount added is in excess of that required by goldfish to account for the maximum expected loss during extrusion which is displayed in table 3 p8. The vitamins will all be added in the water stream to allow for maximal dispersion throughout the particles.

5.0 Processing of Goldfish Feed

5.1 Introduction

The purpose of the experimental work was to find an effective way to produce a feed which satisfies all of the properties which are required for it to be successful. The feed needs to meet all of the nutritional requirements of the goldfish, whilst being palatable, digestible, and able to be processed consistently and effectively. The information gained from the initial literature review suggested that extrusion would be the most likely method to achieve the required physical properties for the goldfish food. Extrusion was the only processing method that was investigated.

The objectives of the experimental work were to:

- Combine all of the ingredients into particles which sink slowly, but do not break apart easily.

5.2 Materials and Methods

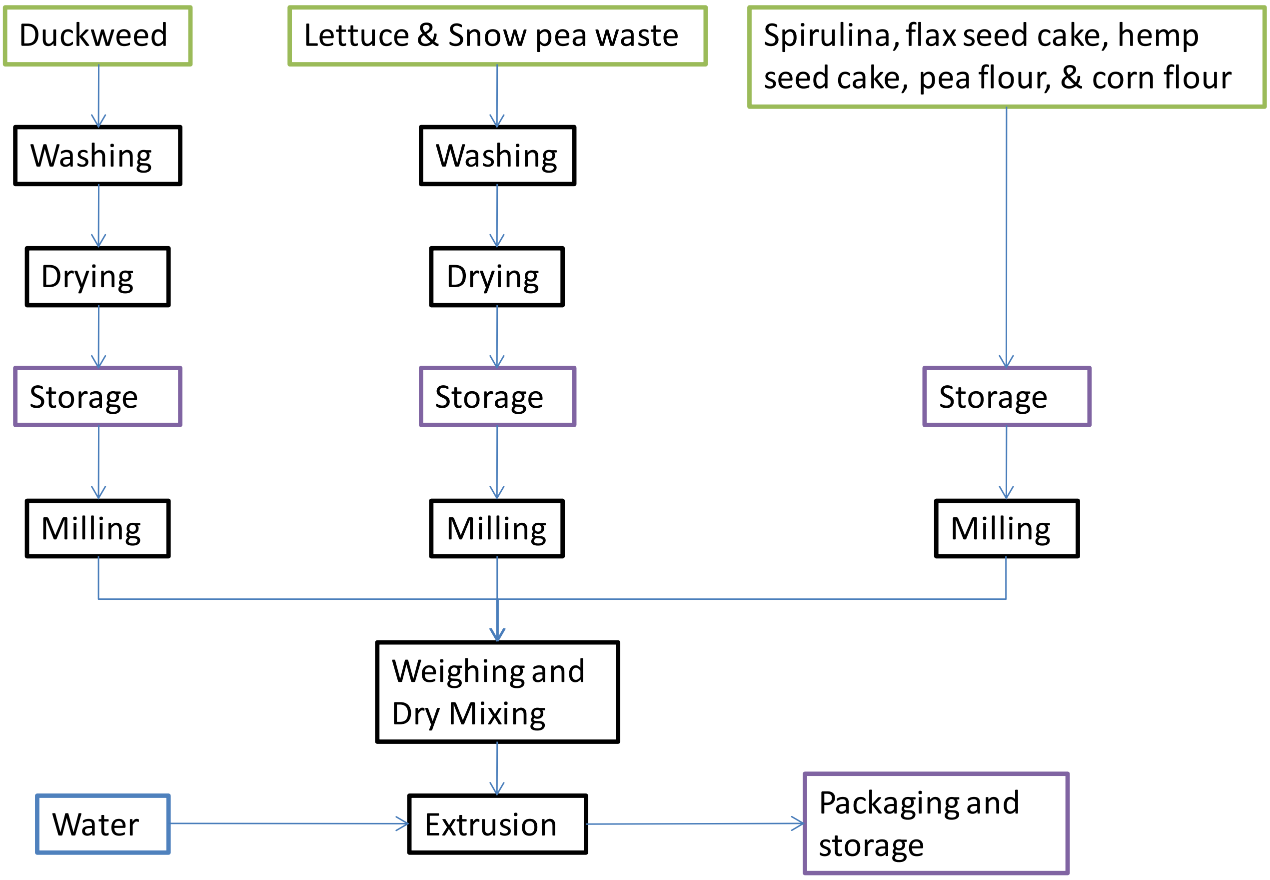

Figure 6: Flow chart of the fish feed making process used during experimental trials.

5.2.1 Washing

The washing step for the duckweed, lettuce waste, and snow pea waste was essential. The duckweed was placed in a fine sieve and held under running water. The purpose was to remove any dead algae from the duckweed because it was unknown if the algae would have been toxic. It was obvious that this method was effective in removing the algae because the algae was evident in the waste water stream. After washing, the duckweed was transferred onto solid drying trays.

The purpose of washing the lettuce and snow pea waste was to remove the dirt because the dirt can destroy the extruder later in the process (G.Radford, personal communication, June 12, 2009). The method used had two stages. Two buckets were filled with clean, cold water and a large handful of lettuce or snow pea waste was dropped into the first bucket. The particulates sank to the bottom and the organic matter remained floating. It was well mixed by hand to ensure that all of the hard grits had been removed and was then transferred to a second bucket of fresh water. It was washed in the same manner and transferred, by hand, onto the mesh drying trays. During the washing, the stems of the lettuce were removed, because they were thicker and took longer to dry.

5.2.2 Drying

After the organic material had been washed, the duckweed was dried on the solid trays, and the lettuce waste or snow pea waste was dried on the mesh trays (as shown in figure 7). The dried use was a Whitlock Speedy Smoke'N'Cooker. The materials were always kept separate when in the drier. They were dried at temperatures not exceeding 80°C for approximately 30 hours to ensure that it had been adequately dried for milling. The dry mass of the duckweed was recorded to determine the moisture losses from it (Appendix 12.5). It was found that the average moisture content of the duckweed was 95.1%. The dry materials were bagged separately, labelled with the dry mass and date, sealed, and stored in a chiller at 4°C until required for milling.

Figure 7: Dryer used during experimental work

5.2.3 Milling

The dry duckweed, dry lettuce waste, dry snow pea waste, flax seed cake, and hemp seed cake all needed to be milled into a powder so that they could be effectively mixed and fed into the extruder. The mill that was used was a Retsch ZM200 (as shown in figure 8), and the screen was 500µm mesh. Each dru ingredient was milled separately and bagged separate so they could be weighed separately in the future. The bags were stored in the chiller at 4°C until required for extrusion.

Figure 8: Mill used to convert all dry materials into powder

5.2.4 Weighing and dry mixing

The purpose of the first extruder trial was to gain a feel for the extruder and how the materials behave through it. The costly ingredients, such as duckweed, spirulina, and vitamins, were not included in the formulation, but were substituted out for similar ingredients instead. The ingredients (as shown in table 5) were dry mixed in a sterilised bucket until all of the different powders appeared to be well dispersed throughout the mixture.

Table 5: Ingredients used in trial runs

Run 1 (25/08/09)		
Component	**Mass (kg)**	
Snow Pea Waste	0.14	
Lettuce Waste	0.34	
Yellow Pea Flour	0.2	
Wheaten Corn Flour	0.28	
Hemp Seed Cake	0.12	
Flax Seed Cake	0.12	
Lettuce to substitute Duckweed	0.57	
Snow Pea Waste to substitute Spirulina	0.24	
	2.01	kg

Run 2 (18/09/09)		
Component	**Mass (kg)**	
Snow Pea Waste	0.14	
Lettuce Waste	0.34	
Yellow Pea Flour	0.2	
Corn Flour	0.28	
Hemp Seed Cake	0.12	
Flax Seed Cake	0.11	
Dry Duckweed	0.57	
Spirulina	0.24	
	2.00	kg

5.2.5 Extruding

The extruder that was used was a Clextral BC21 Twin Screw Extruder (as shown in figure 9). Initially, corn flour was fed through the extruder, so that the settings could be adjusted for some expansion, but without wasting the fish food raw materials. Once it was deemed that the corn flour product was appropriate, the dry powder was emptied into the hopper. The extruder continued producing the corn flour product until the fish food mix started coming through. This took approximately 20 minutes. Once the product was mostly the fish food, with very little residual corn flour, a bowl was placed under the die to collect the product. The barrel temperatures, screw speed, feed rate, and water flow rate were all adjusted using the controls to give a product which was spherical, and was of a density which would sometimes float and sometimes sink when dropped into water. The extruder conditions that were used are shown in Appendix 12.6. The maximum blade speed was always required to cut the product into the smallest possible pieces. The only variables that were altered were the water flow rate into the extruder barrel, the feed rate, and the temperature at the end of the barrel. The water flow rate was increased if the product appeared to be too dry. The feeds were all stored in plastic containers in the chiller at 4°C until required for testing.

Figure 9: Extruder that was used during the trials

5.3 Results and Discussion

Initial indications suggest that the desired properties for a goldfish feed can be achieved using an extruder. Some of the particles would float and the others would sink which is ideal. The extruder conditions that were required are expected to vary each time that it is run, so they will need to be altered to meet the requirements by the product which is being produced. It is essential to have someone who is familiar with an extruder, and who will be able to make decisions about the best way to make alterations to be able to make the appropriate changes while the extruder is being used. Generally, it was found that an increase in the water flow rate would increase the wetness of the product, and therefore, increase its density. It was also found that an increase in the barrel temperature will decrease the density of the particles. This was expected because it increased the amount of evaporation that occurred in the product, and therefore, increased the expansion. Unfortunately, the high temperatures throughout the barrel will degrade many of the vitamins, but they can be added in excess to compensate for this. The vitamin content of the final product will be adequate for fish, but it will add extra costs having to include it in excess.

A mass balance was not performed on the extruder, but it is estimated that approximately the same mass of product was produced as the mass of dry raw materials entering the extruder. Water was added into the barrel via a separate stream but there would have also been wastage due to product being left in the barrel after completion, and wastage due to the product not having acceptable physical properties during the start of the run.

A major issue was found when dealing with the powdered lettuce and duckweed. The dust would get suspended in the air and inhaled. This could be a major health issue if subjected to it for a long period of time. It is recommended that all milling, weighing and mixing, and extruding is to be done only when wearing a face mask.

A possible issue with drying all of the raw materials separately is that they may contain different moisture contents. Since the combination of ingredients is calculated from the dry solids of the different materials, just weighing the powders (including any moisture which is present) will not be adequate. Actual mass of dry solids will need to be known and be used instead.

It was found that the stems of the lettuce in the lettuce waste would not dry as quickly as the leaves because they were thicker. It would be ideal for the materials to be dried uniformly so it is recommended that they are to be removed before drying to achieve this. It was also observed that dirt and sand got stuck in gaps in the stem, so they took longer to wash. Removing the stems will decrease the amount of dirt that will continue through the process which is essential. Dirt and sand will destroy the extruder, so it was essential that they must have been removed before that step of the process (G.Radford, personal communication, June 12, 2009). Removing the lettuce stems, and thoroughly washing the waste lettuce leaves and snow pea waste, did adequately remove enough dirt. This was proven because the extruder ran fine. The major problem with the washing process was the length of time it took. It took an estimated six hours to wash enough lettuce and snow pea waste for six kilograms of the final product. However, the washing was done in a small sink, and could be more efficient with a larger sink.

5.4 Conclusions

- The extruder can effectively produce goldfish food with the required properties.
- Slight alterations need to be made every time that an extruder is run so that the product's properties are exactly what is required.
- It is essential that all dirt is to be removed during washing.
- Waste lettuce stems must be removed before drying.
- Milled lettuce and duckweed powder gets suspended in the air and may be inhaled, so a dust mask will need to be worn to prevent possible health problems.

6.0 Comparison of Goldfish Feed to Current Commercial Products

6.1 Introduction

For a goldfish food to be consumed and digested by the fish, it must have correct floating/sinking properties, and must not be too hard, doughy or powdery (Guillaume *et al*, 2001). No data could be obtained on the ideal properties for a food for goldfish, so it was decided that the feed would be appropriate if it was comparable to other feeds which were currently on the market. All of the properties needed to be similar before the feed could be considered as suitable for goldfish to consume. There are many different goldfish foods on the market which vary in size, composition, shape, and density. Two were selected which had very different properties. All of the different feeds that were produced in the second trial run on the extruder were compared to the commercial feeds.

The objectives of the experimental work were to:

- Obtain quantitative data about the important physical properties of the feeds which had been produced.
- Compare the data to other feeds on the market.
- Conclude if the feed will be suitable for goldfish to consume.

6.2 Materials and Methods

6.2.1 Samples and commercial feeds compared.

Table 6: Samples and commercial feeds used in comparison

Name	Details	Shape	Particle Size
Commercial A	Nutrafin Max - Mostly Fishmeal (47% Protein Min)	Cylinders	ø 3mm, 3-5mm long
Commercial B	Tetracolor - Algae meal and fishmeal (30% Protein Min)	Random	1-3mm wide
Sample 1	Extruder condition A from run 2	Cylinders	ø 2mm, 3-6mm long
Sample 2	Extruder condition A from run 2 but coated in canola oil	Cylinders	ø 2mm, 3-6mm long
Sample 3	Extruder condition B from run 2	Spheres	ø 2-2.5mm
Sample 4	Extruder condition B from run 2 but coated in canola oil	Spheres	ø 2-2.5mm
Sample 5	Extruder condition C from run 2	Spheres	ø 2.5-3mm
Sample 6	Extruder condition B from run 2 but coated in canola oil	Spheres	ø 2.5-3mm

Figure 10: Commercial goldfish foods used for comparison

The two commercial feeds that were used had very different compositions to each other. One had fishmeal as the main protein source, and the other had algae meal (probably spirulina) and fishmeal as the protein sources. All of the samples had spirulina and duckweed as the main sources of protein. Only the samples from the second extruder run were used, because product from the first extruder run did not have all of the correct ingredients. Samples from each set of extruder conditions were used, and they were tested as they were, or with a coating of canola oil on them.

6.2.2 Floating and sinking properties

The floating and sinking times were simply measured by dropping the particles, one at a time, into a 30cm column of water. Using a split timer, the floating times and sinking times were recorded. Each sample or commercial product was measured five times, then the water was replaced, and another sample would be measured five times. Thirty measurements for the commercial products were taken, and twenty for each of the samples. The results are shown in appendix 12.7.

6.2.3 Sieving to determine crumbliness and particle size distribution

The crumbliness was assessed by sieving approximately 100g of samples 3 and 5 and 100g of each commercial product for five minutes. Only samples 3 and 5 were used because they were the preferred ones from the floating/sinking analysis. The sieves used, in descending order, were 850µm, 600µm, 355µm, 212µm, and the solid bottom tray. The particles were weighed to the nearest 0.01g and placed on top of the 850µm mesh sieve and the lid was put on top. It was secured to the shaker, and each sample was shaken at the same intensity for five minutes. The trays/sieves had been weighed beforehand, and were weighed and recorded after the sieving (as shown in appendix 12.8). The trays and sieves were cleaned with a compressed air gun before the next sample was run.

6.2.4 Hardness determination

The hardness of the commercial products, and samples 3 and 5, were assessed using a TA-XT2 texture analyser (as shown in figure 11). Individual particles were soaked in water for 0s, 30s, 60s, 90s, 120s, 180s, 240s, 300s, 360s, and 480s and transferred onto the plate and crushed by a flat bottomed probe. The force was recorded, and the maximum peak was hardness measurement for that particle. Quadruplicate measurements were taken for each sample at 0s, 120s, and 300s. The particles were soaked by simply dropping them into a tray of water, and leaving them for the set time, and removing them with tweezers without crushing them. Qualitative observations were also made about the hardness and crushing sounds, and are shown in appendix 12.9.

Figure 11: TA-XT2 texture analyser with flat bottomed probe

6.3 Results and Discussion

Samples 1 and 2 had an elongated shape compared to the other samples because the blade could not cut it fast enough to produce spheres. The size of all of the sample particles were an intermediate of the commercial product particles. This suggests that the feed is of a size which could be consumed by goldfish. Samples 5 and 6 were larger spheres than samples 3 and 4, so it suggested that more expansion had occurred, so it would have been expected that those would have been less dense, and would have been more likely to float. This observation was supported by the floating data which was obtained (as shown in figure 12).   The floating properties of the goldfish food are quite important because some fish feed from the surface and others while the particles are sinking (P.Davie, personal communication, March 27, 2009). No data could be found on the ideal floating time or sinking velocity of the particles so the samples were compared with the commercial feeds.

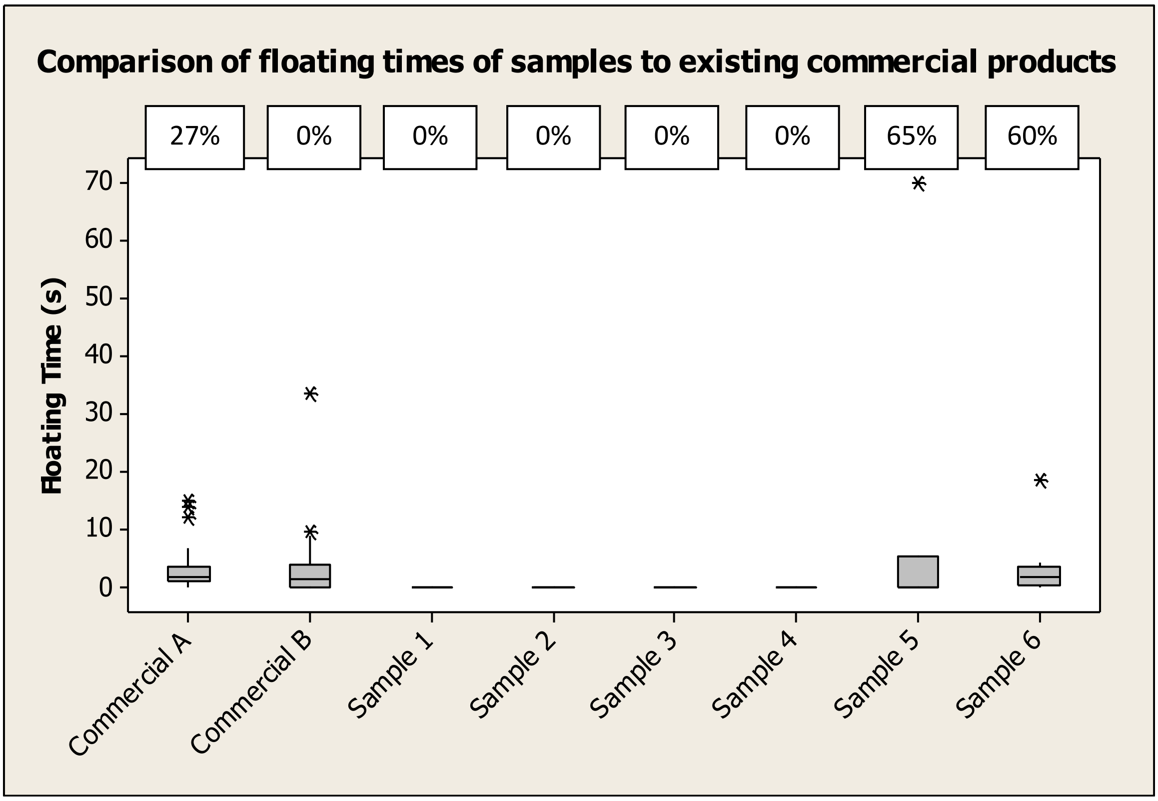

Figure 12: Floating time distribution of the feed particles

The percentage above each box plot refers to the number of particles from that sample which remained floating for more than two minutes. Only the particles that sank were plotted on the box plot because the floating particles skewed the data and gave box plots that were difficult to interpret. Two minutes was used as the cut off time because all of the feed should have been consumed by the goldfish in that time, as recommended on the packaging of each product. 27% of commercial A remained floating after two minutes and 0% of commercial B did. It suggests that it should be aimed that approximately 1/5 particles of the feed produced should float for more than two minutes. It could be achieved in two ways; get the extruder conditions perfect so that 20% will float for two minutes and the rest will have sunk in that time, or produce 80% on one extruder condition set to sink, and 20% on another extruder condition set to float and mix them.

Both of the commercial samples showed a more broad distribution of floating times than all of the trial samples. A broad distribution of floating times is useful because it causes the particles to sink at different times, so the fish are less likely to miss sinking particles. Particles which sink to the bottom, and do not get consumed by goldfish, can have negative affects to the tank, such as nitrogen leaching which promotes algal growth (A.Hardacre, personal communication, May 8, 2009). The particles for samples 5 and 6 which did not float for more than two minutes, showed a floating time distribution similar that of the two commercial feeds, so either of them could be suitable.

The sinking velocity (which can be compared between samples by measuring time instead) of the particles is important because the goldfish will feed in falling particles. The particles need to fall fast enough to visually stimulate the fish but must not be so quick that many will land on the bottom of the tank and not get consumed. The sinking time distribution of the samples is shown in figure 13.

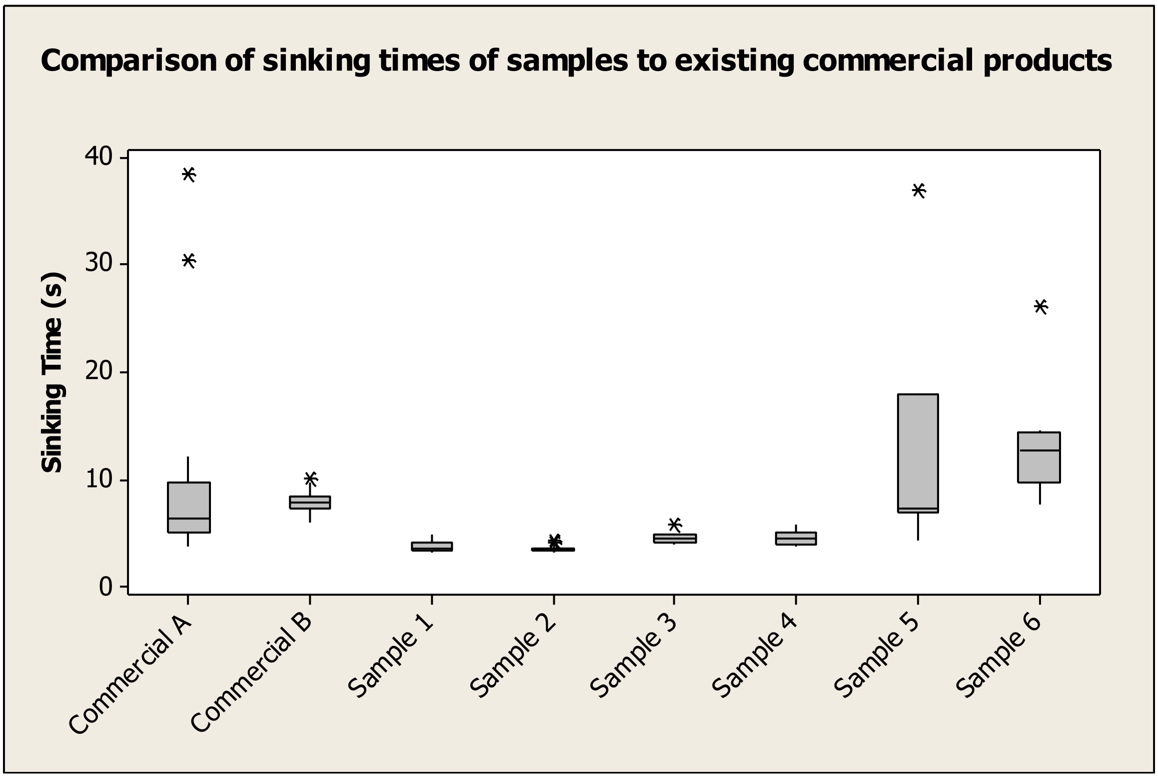

Figure 13: Sinking time distribution of the feed particles

Samples 5 and 6 both showed average sinking times which were greater than that of the commercial products, but samples 1,2,3, and 4 all had average sinking times which were less than that of the commercial products. This suggests that it will be possible to get a set of extruder conditions which will produce particles with sinking time distributions that are very similar to that of the commercial products. If this can be achieved, it is assumed that goldfish will take the food into their mouths. If the feed tastes correct to the fish, they will then ingest it (P.Davie, personal communication, March 27, 2009). A mixture of sample 4 and 6 is estimated to be good, but a mixture of samples 3 and 5 should also be adequate. Samples 1 and 2 are of a poor shape, and have poor floating/sinking distributions so were not continued with any comparison testing. Samples 4 and 6 had added oil, which is unfavourable nutritionally, and could lead to quality issues due to lipid oxidation. Since samples 3 and 5 were close to the required properties, they were continued with further comparison testing.

The goldfish eat the whole food particles, so it is important to produce a food which is not too crumbly (Guillaume *et al*, 2001). If crumbling occurs, the small crumbs will not be consumed by the goldfish, which can lead to nitrogen leaching and algal growth (A. Hardacre, personal communication, May 8, 2009). A sieve analysis was performed because it simulates transport by vibrating and it separates the particles out simultaneously. The particle size distribution is presented in figure 14.

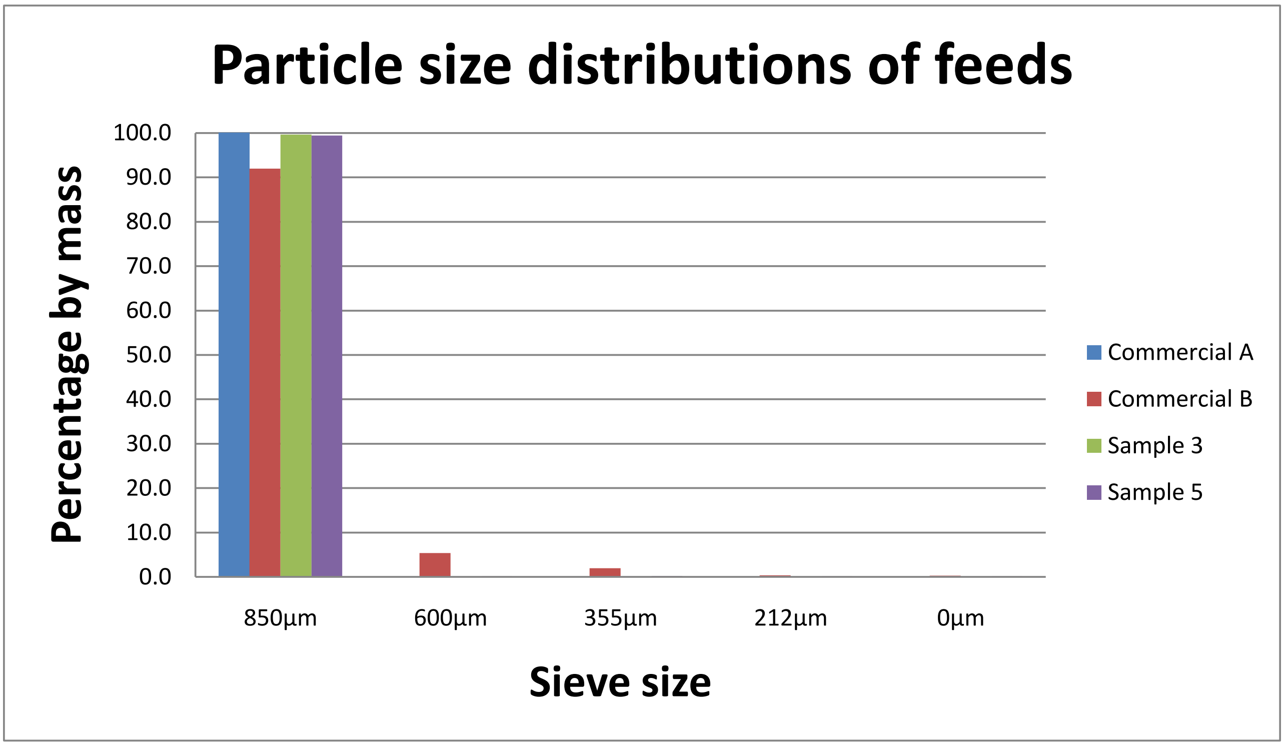

Figure 14: Particle size distribution of different samples

It is obvious that sample 3 and 5 have very similar particle size distributions to the commercial A product because almost 100% of each sample is retained above the 850µm mesh sieve. Commercial B is the only feed which differs substantially. Only 92% of the feed was retained on the 850µm mesh. 5.4% and 2% were on the 600µm and 350µm mesh' respectively. This distribution is probably due to the random shape and small size of the commercial B particles, rather than it being crumbly. Sample 3 and 5 are both expected to be suitable in regards to the size and adequate binding of the particles.

The final comparison test that was performed was a texture analysis to measure the hardness of the samples after being soaked in water for varying lengths of time. The purpose of soaking them in water was to simulate the moisture absorption that will occur in the fish tank before the feed is consumed by goldfish. A plot of the forces required to deform the particles is presented in figure 15.

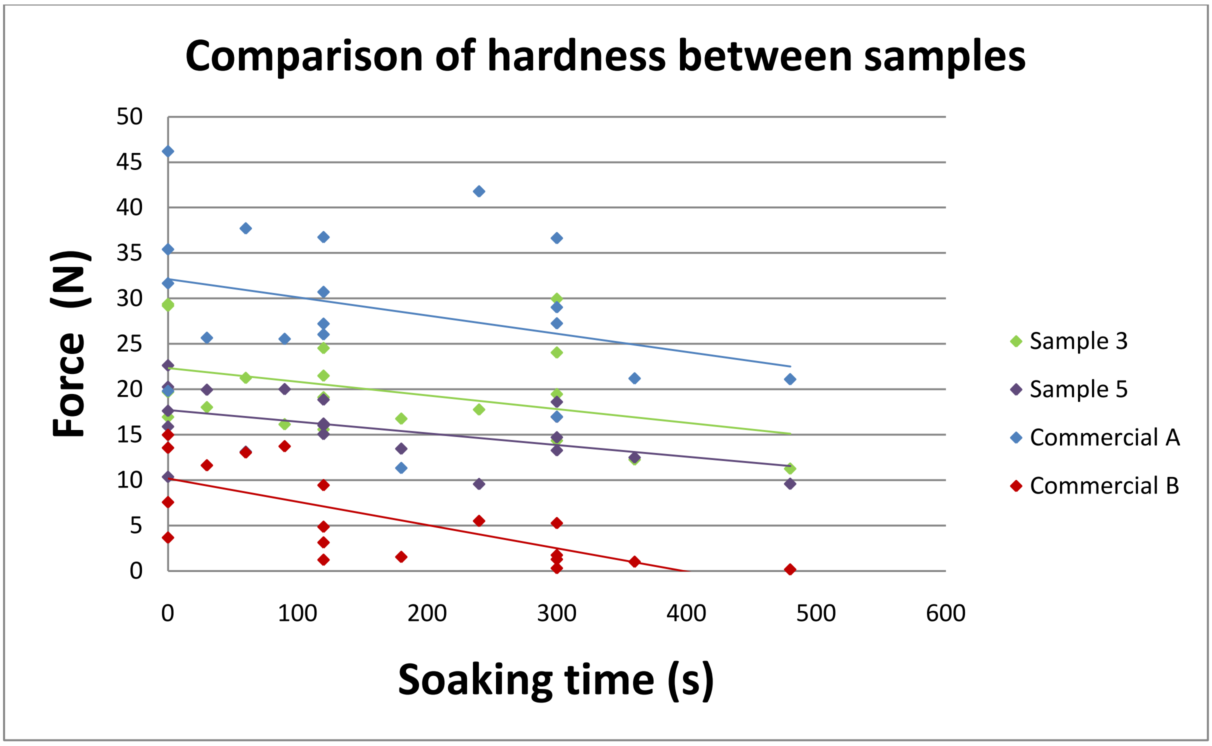

Figure 15: Plot of hardness against soaking time for the different feed samples

The hardness of each of the samples follows the same trend as the commercial products. The hardness of samples 3 and 5 are between that of the two commercial products, which indicates that the fish will probably be able to ingest and digest the feed. All of the feeds soften at a similar rate in the water, which suggests that the food will break up in a similar fashion in the digestive tract of the goldfish. Qualitative data was also recorded (Appendix 12.9) because it can be used to assess if the deformation is due to the particle shattering or getting crushed. As more water is absorbed, the particles become softer so the maximum force is due to squashing, rather than shattering. Commercial A made a cracking noise after 0s, 120s, and a very faint noise after 300s soaking. Commercial B made a cracking noise after 0s, but none after 120s of soaking. Sample 3 made a cracking noise when crushed after 0s and 120s of soaking, but not after 300s of soaking. Sample 5 made a cracking noise after 0s, very faint cracking noises after 120s, and no cracking noise after 300s of soaking. Sample 3 and 5 are both between the two commercial products, so both are expected to soften at a suitable rate for goldfish to safely consume.

6.4 Conclusions

- Samples 3, 4, 5, and 6 are expected to be of a suitable size and shape for goldfish to consume.
- The floating times of the samples produced were not ideal, but it is probably that it can be achieved with the extruder.
- A mixture of samples 3 and 5 will have a sinking time distribution similar to that of current feeds on the market.
- The particle size distribution of samples 3 and 5 is similar to one of the commercial feeds and is deemed to be suitable and not too powdery.
- Samples 3 and 5 are believed to be of an adequate hardness to be suitable for goldfish to consume.
- Samples 3 and 5 soften at a similar rate to current commercial feeds on the market.
- A mixture of samples 3 and 5 is believed to be suitable as a complete goldfish feed.

7.0 Testing of Fish Feed on Goldfish

7.1 Introduction

Testing of the goldfish feed on goldfish has not yet been performed due to time restrictions. It must be completed before the product is to be sold commercially, and it is recommended to be done before any other investments are to be made. It is essential, from a financial and moral perspective, to confirm that the feed is safe for adult goldfish to consume as their complete food for their lifetime. It is recommended that the fish food should be fed to the fish for a three month period and the growth rates and mortality rates should be monitored (P. Davie, personal communication, March 27, 2009).

The objectives of the study will be to:

- Determine if there is a significant difference between the mortality rate and growth rate of goldfish which are fed a control diet or the sustainable sample diet.

7.2 Materials and Methods

7.2.1 Feeds

The control feed is recommended to be tetracolor sinking goldfish granules, which was used as 'commercial B' during the comparison of the produced product to current products.

The sustainable sample feed will be provided, and will be similar to 'sample 3' and 'sample 5' from the previous testing.

The first step should be to feed the test food to about five goldfish for one week and observe them. If the goldfish have been observed ingesting and excreting the food, then complete trial should proceed. If they do not ingest the feed, then they are likely to die of starvation, and if they do not excrete the feed they will also die.

7.2.2 Tanks

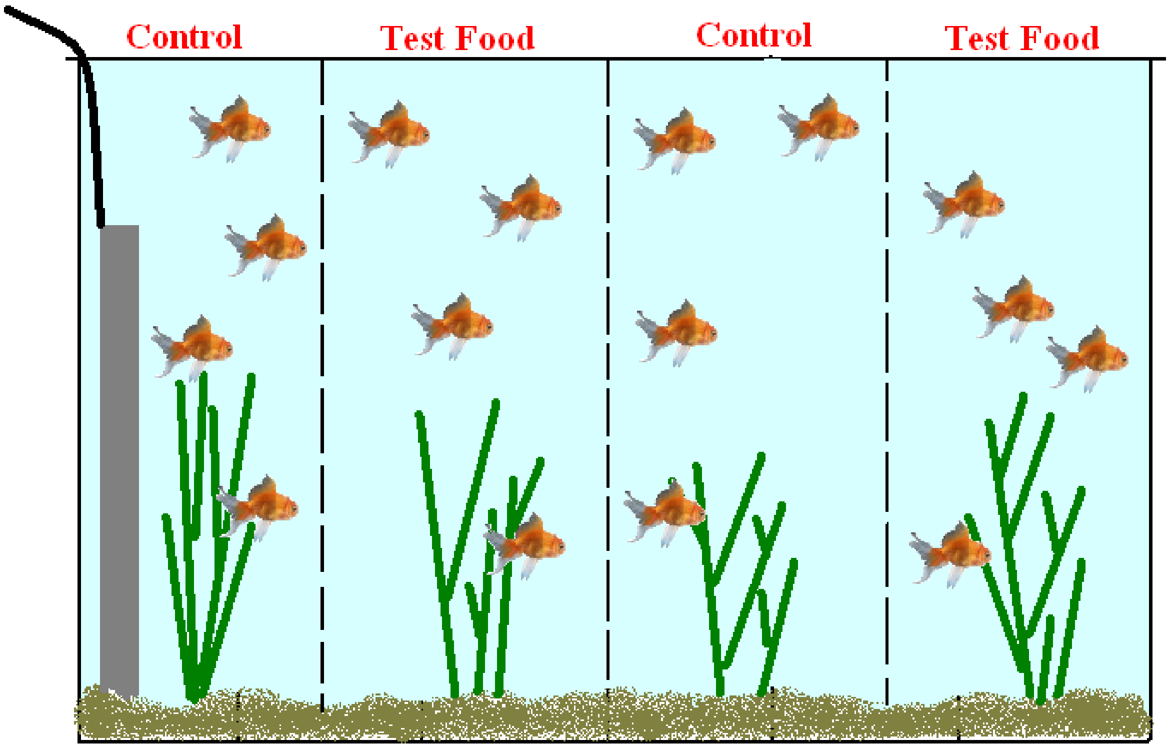

Figure 16: Recommended fish tank set up for testing goldfish food

It is recommended that five fish tanks are to be used, with each tank housing sixteen fish. The tanks should be of a size which is suitable for sixteen goldfish as recommended by a pet fish professional. It is recommended to divide the tanks into equal sized divisions, and each division will receive the control diet or the test diet for the entire experiment. The purpose of having divisions in the tank is to reduce the effect of any variables, such as water temperature or nutrient content because the fish on both diets will be subjected to those conditions. The mesh which divides the tank must not have a mesh size exceeding 1mm. This will prevent any feed from passing through to the fish on the other diet.

It is recommended that the fish tanks are to be similar to that of real fish tanks to simulate the environment which the fish will actually be fed in. This includes aquatic plants and stones. It will allow for the goldfish to eat any plant material or algae which they would normally consume in a fish tank. The aquatic plants which are added must be present in similar amounts in each of the divisions for a given tank.

7.2.3 Fish

It is recommended that eighty goldfish are to be purchased. The goldfish should all be of a similar size, but cannot be juveniles because juvenile goldfish require a greater protein content in their diet (National research council, 1993). Young, mature fish are recommended. It is recommended that the goldfish are to be of a range of colours and markings so that they can easily be identified. Four different looking fish should be placed in each division, and a description of that fish should be recorded (for example: "Fish #58 (Test diet), Tank 4, Division 3, Orange fish with black and white spots"). All fish must be easily identifiable but must also be of the *Carassius auratus* species.

All eighty goldfish must be weighed before placing them into the tanks. It is recommended that a scale is to be 'zeroed' with a container of water from the fish tank on it. The goldfish can then be removed from the tank with a net, allowed to drip dry briefly, and placed into the container of water and weighed. The fish must be introduced into the tanks in a manner suggested by a pet fish professional. The weights of the goldfish will need to be taken weekly for the entire three months and entered into a spreadsheet. It is recommended that sixteen (ie one tank) are to be measured each day and recorded, so all eighty can be measured in each five day period, with two days off after the fifth day. The total weight of each fish will need to be recorded in a spreadsheet and its weight gain will need to be calculated.

7.3 Analysis of the data

Once the weight gain of each fish for each week has been calculated, a plot can be made of the total weight gain since the start of the experiment against the number of days since the start, as shown in figure 17.

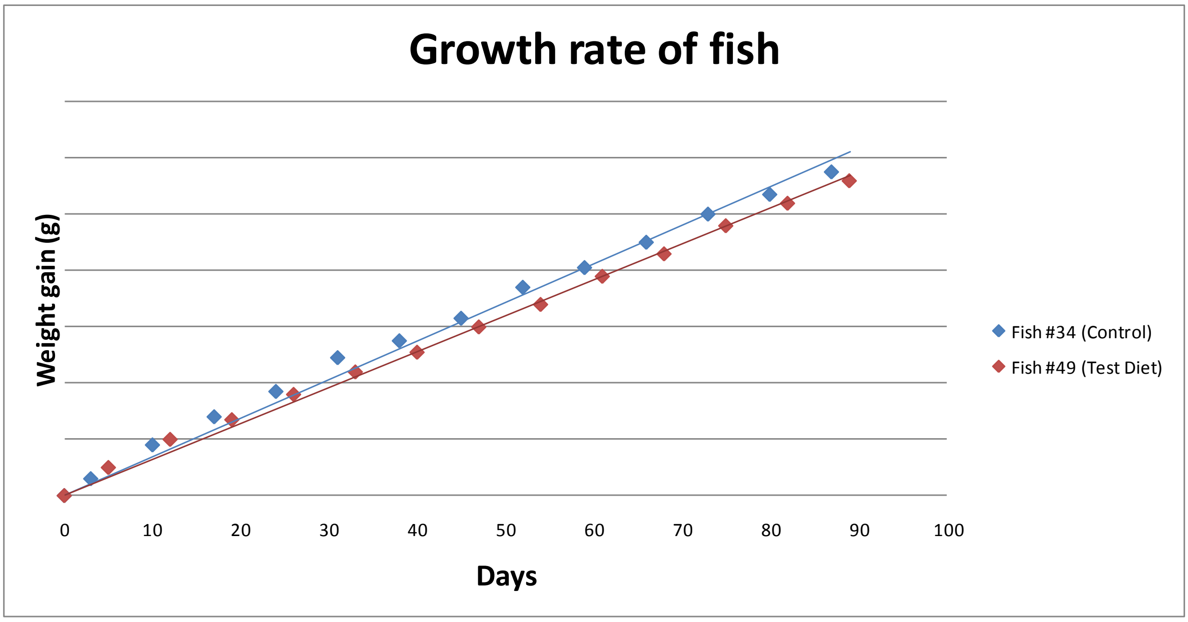

Figure 17: Example of a plot of the growth rate of goldfish

The gradient of the graph will need to be obtained for every goldfish, which is the growth rate in g/day. The growth rates of each individual fish can be used to predict if there is a significant difference between the two feeds using a 2-Sample T-Test and Confidence Interval on Minitab (found under the Stat > Basic Statistics tab). If the 95% confidence interval contains zero, then there is no significant difference. The difference in mortality rate between goldfish on each feed can be found using a 2 Proportions Test and Confidence Interval (also found under the Stat > Basic Statistics tab). Again, if the 95% confidence interval contains zero, then there is no significant difference. The spreadsheet should be set up as shown in figure 18.

Worksheet 1 ***

↓	C1-T	C2-T	C3	C4	C5	C6
	Fish Code number	Feed Type	Growth rate (control)	Growth rate (test)	Mortality (control)	Mortality (test)
1	#1	Control	0.132	*	0	*
2	#2	Control	0.126	*	0	*
3	#3	Control	0.145	*	1	*
4	#4	Control	0.113	*	0	*
5	#5	Test	*	0.088	*	0
6	#6	Test	*	0.117	*	0
7	#7	Test	*	0.136	*	0
8	#8	Test	*	0.133	*	0
9	#9	Control	0.096	*	0	*

Figure 18: Example of how the spreadsheet could be set out to analyse the growth rate and mortality rate on the different feeds

A '1' should be entered in the mortality column if that that fish died during the course of the experiment, or a '0' if it did not.

NOTE: The values of the example data are arbitrary and the actual data may vary from it greatly.

If there is no significant difference between the growth rates or the mortality rates of the test and control, then the feed will be suitable to send to market.

8.0 Recommended Protocol

8.1 Introduction

8.2 Large scale duckweed production

Obviously, different practices will need to be implemented when the duckweed is grown in a large outdoor pond to the practices used in the trial. The temperature and amount of sunlight are difficult to alter, but the amount of water movement could be reduced. Wind can blow the duckweed towards the sides of the ponds, which reduces the growth of the duckweed (Skillicorn *et al*, 1993). It is recommended that wind breaks are to be situated around the edge of the pond to reduce the wind effects as much as possible. The wind breaks could be trees or netting. It may also be beneficial to have surface barriers to divide the pond into grids (Skillicorn *et al*, 1993). The result is that the duckweed remains more spread out across the pond when there is wind.

It is recommended to harvest the duckweed every day and from different areas of the pond. The duckweed should be harvested with a mesh or net which is to be slid under the surface and lifted out with the duckweed on top. A method will need to be developed to collect duckweed from the centre of the pond without disrupting the surface coverage. This will be difficult or expensive to achieve with the current pond dimensions (approximately 20m x 20m square). A system of pulleys which can dip a mesh into the duckweed at any point in the pond may be the most likely operation to achieve the required harvesting. It is recommended that the duckweed is to be dried during the same day that it is harvested.

Ideally, the duckweed should be grown in narrow, shallow ponds with an area to walk between them, like that in figure 19. This will allow for effective harvesting and will better utilise the volume of water since the duckweed only grows on the surface.

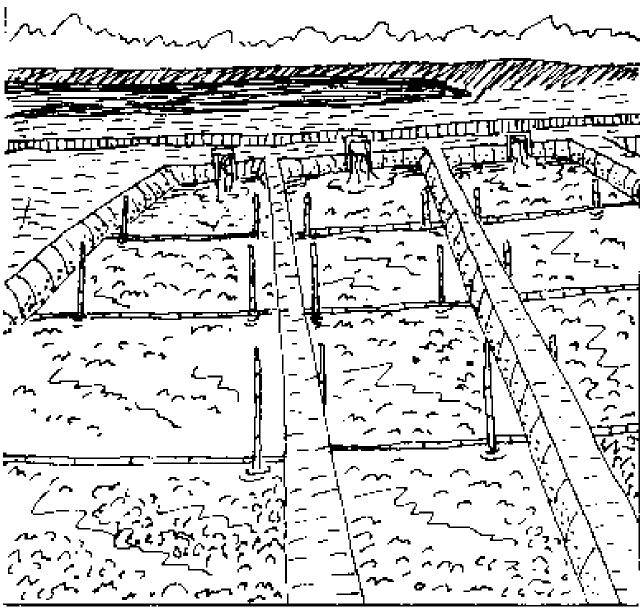

Figure 19: Ideal set-up for growing duckweed (from Figure 7 of Skillicorn *et al,* 1993)

8.3 Production of goldfish feed

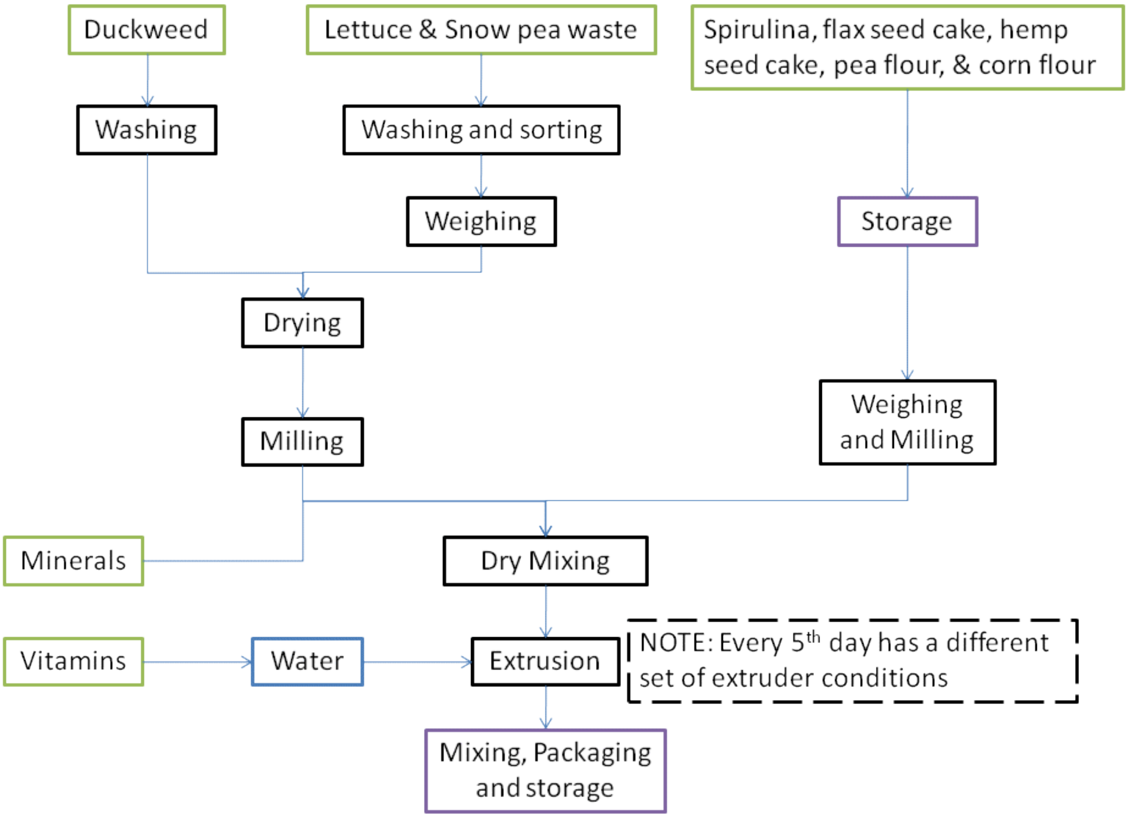

Figure 20: Recommended process for large scale fish food production

The major differences between the recommended process and the one that was used during the trials are in the weighing and drying of the ingredients, and the inclusion of vitamins and minerals. It is recommended that the amount of lettuce waste and snow pea waste is to be decided on a fresh basis, as opposed to dry mass. This means that the total solids entering the drying step for each ingredient is in the same ratio as is required in the final product. If the fresh ingredients are all mixed and dried together, the actual degree of drying is not important, provided that it is dry enough to be effectively milled and dry mixed with the other ingredients. It has been estimated that it will be adequate if the materials are dried to a moisture content of no greater than 10%. The other dry powders will need to be dry mixed in, based on the initial mass of fresh duckweed. The vitamins are recommended to be dissolved in the water and fed into the extruder barrel to provide maximal dispersion through the product.

8.3.1 Recommended washing and sorting process

8.3.1.1 Duckweed

It is recommended that the duckweed is to be washed in the same fashion as during the experimental trials. It should be placed on a fine sieve and washed with fresh, running water. After being washed, it should be transferred onto large solid drying trays and weighed. The weight of the trays will need to be deducted so only the fresh duckweed mass is included. The total mass will need to be entered into the 'Mass of duckweed harvested' cell (I2), in the 'Calculations' tab of the excel spreadsheet which is named 'Formulation.xls'. This spreadsheet is displayed as figure 21.

NOTE: Only the orange cells should have data entered directly into them. The grey cells should only have the 'solver' function performed on them. All of the other cells must not be changed.

Mass of duckweed harvested	35	kg

FRESH INGREDIENTS TO ADD:		
Snow pea waste (fresh)	2.09	kg
Carrot waste (fresh)	0.00	kg
Lettuce waste (fresh)	20.74	kg
Fresh moisture content	*94.5*	*%*

Sample mass into dryer	3	kg
Maximum allowable mass out	**0.183**	kg
Sample mass out of dryer	0.180	kg
Dry moisture content	*8.5*	*%*

Mass of dry solids into dryer	3.176	kg
Maximum allowable moisture content	**10**	*%*

Use solver function on this cell to make it equal 10% by changing the 'maximum allowable mass out cell'

DRY POWDERS TO ADD:		
Spirulina	0.802	kg
Pea flour	0.765	kg
Corn flour	1.000	kg
Hemp seed cake	0.381	kg
Flax seed cake	0.349	kg
Monobasic Calcium Phosphate	93.2555	g
Zinc Sulphate	0.6217	g

Mass of dry solids into extruder	6.217	kg
Mass into extruder excl vitamin water	6.768	kg
Mass of water required for vitamins	0.548	L

DRY VITAMIN POWDERS TO DISSOLVE IN WATER:		
Riboflavin Universal	0.0207	g
Calcium D-pantothenate	0.4352	g
Dry Vitamin E 50% CWS/S	3.8856	g
D-Biotin	0.0187	g
Ascorbic Acid	14.3470	g
ROCOAT® Niacinamide 33⅓%	0.2072	g
Pyridoxine Hydrochloride	0.0555	g

Figure 21: Spreadsheet which should be used to calculate the mass of all ingredients to be added

If the mass of fresh duckweed is entered into this spreadsheet, it will generate the mass of fresh snow pea waste and lettuce waste, as well as all dry ingredients, which will need to be incorporated into the product.

8.3.1.2 Lettuce and snow pea waste

Once the mass of duckweed has been entered into the spreadsheet, the snow pea waste and lettuce waste will need to be washed and weighed. The amount which will be needed of each fresh ingredient will be displayed in the spreadsheet.

It is recommended that a large wire mesh with a sieve size of approximately 5mm is to be used. This size will allow all sand and dirt to pass through but very little of the organic matter to. It should be held underwater and shaken, with the lettuce and snow pea waste above it, until all of the dirt has passed through. The lettuce and snow pea waste should be washed separately. Some unwanted organic matter will remain, such as small pieces of tree bark that were in the soil, but these will be in a minimal quantity and can be affectively milled and extruded. During the washing of the lettuce, all of the stems must be removed into a waste container. This is a crucial step in the process.

Once the snow pea waste and lettuce waste have been washed and correctly weighed out, they should be placed directly on the duckweed drying trays, so that each tray has an equal proportion of duckweed, snow pea waste, and lettuce.

8.3.2 Drying

All of the trays of the fresh ingredients will need to be dried at a temperature of 70°C to a moisture content of less than 10%. It is unknown how long that it will take, but it is estimated to be approximately 20 hours. It will need to be determined approximately how long it takes to dry it to determine what time the trays need to enter the drier on the previous day.

Before any trays have been put into the drier, one tray needs to be weighed and entered into the 'sample mass into dryer' cell on the spreadsheet. This mass must be in kilograms, and must not include the mass of the tray. The contents of the tray must also be representative of the actual proportions of the ingredients. After the mass has been recorded, the trays can be loaded into the dryer. The tray which was weighed must be identifiable because it will need to be reweighed throughout the process.

While the trays are in the dryer, the maximum mass which the tray must be dried to can be calculated. This is found by highlighting the 'maximum allowable moisture content' cell and then using the 'solver' function (found under the 'data' tab). If the solver function is unavailable, Excel help can explain who to add it in. The target cell (M13) must be set to equal the value of 10 by changing the 'maximum allowable mass out' cell (I11) and pressing 'solve'. The tray must be dried until all of its contents weigh less than the value stated in the 'maximum allowable mass out' cell. The actual mass of dried material on the tray will need to be entered into the 'sample mass out of dryer' cell (I12). Once the materials are adequately dried, all of the trays can be removed from the dryer and taken to milling.

8.3.3 Milling

The amount of spirulina, hempseed cake, and flax seed cake that will be needed will be displayed on the spreadsheet (cells I16, I19, and I20 respectively). It can be weighed out and milled with the dried lettuce, snow pea waste and duckweed. It is recommended to mill it to a maximum particle size of 500μm to allow for effective extrusion. All of the ingredients can be milled together, instead of keeping them separated, because they are going to be dry mixed anyway. The mill should be cleaned after each run of that day.

NOTE: a dust mask must always be worn during milling and handling of the milled products to prevent the duckweed and lettuce powder from being inhaled.

8.3.4 Dry Mixing

The milled materials will need to be dry mixed with the pea flour, corn flour, monobasic calcium phosphate, and zinc sulphate in a large container. The mass required of each of the ingredients will be calculated automatically and displayed in the spreadsheet.

8.3.5 Preparing vitamin water

Before the extruder is ready to be run, the vitamin water must be prepared. All of the vitamin powders must be weighed dry, mixed dry and then dissolved in water by thoroughly mixing it. The amount of cold water to use will be presented in the 'Mass of water required for vitamins' cell of the spreadsheet.

8.3.6 Extruding

NOTE: It is recommended to run the extruder on one set of conditions for the first four days of the week, and on another set on the fifth day of that week, as explained in paragraph 2.

8.3.6.1 Setting up the extruder

It is recommended to start the extruder with a corn flour and plain water mixture, until it is running smoothly and some expansion is evident. This will reduce the amount of wastage of the goldfish feed ingredients. The extruder must be allowed to heat up to similar temperatures throughout the barrel as were used in the trial runs (as shown in Appendix 12.6). The other variables (screw speed, feed rate, water flow rate, and blade speed) will all need to be adjusted accordingly to produce the correct properties required by the product. It is recommended that a person with prior extruder experience is to be used to adjust the variables, so an approximate value for each variable can be found. Once this is known, someone will need to know how to make minor adjustments to the extruder conditions to change the properties of the product.

8.3.6.2 Running the extruder

Once the extruder is running steadily on the corn flour mixture and the amount of corn flour in the hopper is low, the dry mix can be poured into the hopper. When the fish food product first starts to pass through the die hole, the vitamin water can be added to the water feed. It is recommended to have kept the amount of water in the water container low, but closely monitored, during the corn flour extrusion to prevent too much dilution of the vitamin water upon addition of it. The blade speed should be set so that the particles are cut into spheres. The feed rate, screw speed, and water flow rate must be adjusted, until the particles produced just sink when dropped into water (on the fifth day of the week, the variables can be adjusted so that the particles float). The floating/sinking can be assessed visually. It is recommended to adjust the feed rate to change the product properties, because it was found during the experimental trials that the feed rate has an effective control over the properties. Once the product has the required floating/sinking properties, a bag or container can be placed under the die to collect the product. The floating/sinking properties should be assessed at regular intervals, and if they are not acceptable the appropriate adjustments should be made.

Obviously the amount of water which is required for extrusion will vary for each run, so the amount of water in the container must be carefully monitored. If it is close to running out, then it can be topped up with fresh water.

8.3.6.3 Finishing extrusion

Once most of the dry mix has been used, some more corn flour can be added to the hopper. When this starts to exit the barrel through to die holes, the collection container should be removed, and the heating in the extruder turned off. More water can be added to the water container, and the water flow rate should be turned up, so that a runny mixture passes through. This will reduce the amount of cleaning that will need to be done. Once the corn flour has been used, the feed can be turned off, and the pieces removed and washed. The barrel will need to be removed, and cleaned thoroughly with a wire brush. The screw will also need to be cleaned with a wire brush. All of the parts of the die holes and blade will need to be washed with soapy water. After each run, the extruder will need to be washed and sanitised.

9.0 Costing and Feasibility

9.1 Capital costs

The capital costs are expected to be approximately $257,000, as shown in table 7.

Table 7: Expected capital costs for a new plant

Capital Costs		
Duckweed Ponds:		
	Wind Breaks	$5,000
	Harvesting equipment	$2,000
	Surface barriers	
Factory		$100,000
Dryer		$10,000
Mill		$10,000
Extruder		$120,000
Other equipment (scales, trays, hoses etc)		$10,000
		$257,000

The bulk of the costs are in the plant and the extruder. The extruder is expected to cost approximately $120,000 (A. Hardacre, personal communication, October 27, 2009), but there will be excess capacity on it. To recover some costs, it may be beneficial to find another product which can be extruded while it is not being used.

The capital costs for the duckweed pond will be approximately $7000, but will be dependent on the harvesting method which is implemented.

9.2 Raw material costs

The raw material costs are expected to be approximately $24.12 per kilogram of product produced, as shown in table 8.

Table 8: Expected raw materials costs of goldfish feed

Raw materials	Amount (kg component needed /kg feed produced)	Price ($/kg component)	Price ($/kg feed produced)	Supplier
Snow Pea Waste	0.379	$0.00	$0.00	New Zealand Fresh Cuts
Carrot	0.000		$0.00	New Zealand Fresh Cuts
Lettuce	3.265	$0.00	$0.00	New Zealand Fresh Cuts
Duckweed:	5.510			
Nutrients		$0.00	$0.00	Grown
Land		$0.00	$0.00	Grown
Harvesting Labour		$2.05	$11.30	Grown
Maintenance		$0.50	$2.76	Grown
Spirulina	0.126	$75.00	$9.47	Lifestream
Pea flour	0.119	$0.65	$0.08	Midlands / Gourmet Greens and Seeds
Corn Flour 600 spec	0.157	$0.65	$0.10	Corson Grain
Hominey	0.000		$0.00	Corson Grain
Hemp Seed Cake	0.060	$2.00	$0.12	Oil Seed Extractions Ltd
Flax Seed Cake	0.061	$1.00	$0.06	Oil Seed Extractions Ltd
Riboflavin Universal	0.000007	$280.50	$0.00	Invita NZ Ltd
Calcium D-pantothenate	0.00006	$59.00	$0.00	Invita NZ Ltd
Dry Vitamin E 50% CWS/S	0.0008	$170.00	$0.14	Invita NZ Ltd
D-Biotin	0.000003	$15,000.00	$0.05	Invita NZ Ltd
Ascorbic Acid	0.001	$50.00	$0.05	Invita NZ Ltd
ROCOAT® Niacinamide 33⅓%	0.00002	$62.00	$0.00	Invita NZ Ltd
Pyridoxine Hydrochloride	0.00001	$135.00	$0.00	Invita NZ Ltd
Monobasic Calcium Phosphate	0.015		$0.00	
Zinc Sulphate	0.0001		$0.00	
		Total Cost:	$24.12	/kg feed produced

It can be seen that the only two ingredients which are major contributors to the cost of the raw ingredients are the duckweed and the spirulina. This is expected because they are both in high proportions and are essential for their protein contents. The other ingredients are almost insignificant in comparison. The costs of duckweed are based almost entirely on the expected labour costs involved. The labour costs have been estimated as costing $72 (two labour units at $18/hour for two hours), and have been divided by the estimated 35kg that will be harvest to find the cost if fresh duckweed. The snow pea and lettuce waste have been assumed to cost nothing because they are considered as waste products. The price of the different flours, seed wastes, and vitamins have been found by contacting suppliers of them.

9.3 Expected Profit Margin

The expected profit margin varies greatly with the amount of duckweed available. As the amount of duckweed harvested increases, the profit margin improves. This is because the throughput is increased with increasing the labour or operating costs too greatly. The estimated profit margin with different amounts of product produced is presented in table 9.

Table 9: Expected net profit

Mass of feed produced per day	7	10	20	30	50
Area of duckweed required (m^2)	433	619	1238	1857	3096
Raw Materials (per day)	$168.87	$241.25	$482.49	$723.74	$1,206.23
Labour (per day)	$216.00	$288.00	$360.00	$432.00	$540.00
Overheads (per day)	$30.40	$43.42	$86.85	$130.27	$217.12
Distrubution (per day)	$19.93	$28.47	$56.93	$85.40	$142.33
Ex Factory (per day)	$435.20	$601.14	$986.27	$1,371.41	$2,105.68
Ex Factory (per kg)	$62.17	$60.11	$49.31	$45.71	$42.11
Net profit margin (per kg)	**$19.88**	**$21.94**	**$32.74**	**$36.34**	**$39.94**
Net profit margin (%)	**24.2%**	**26.7%**	**39.9%**	**44.3%**	**48.7%**
Retailer's margin (per kg)	$82.05	$82.05	$82.05	$82.05	$82.05
GST (per kg)	$106.67	$106.67	$106.67	$106.67	$106.67
Retail Price (per kg)	$120	$120	$120	$120	$120

The raw materials per day were calculated by multiplying the cost per kilogram of product by the amount of product expected to be produced in that day.

The labour was estimated to be two-three labour units at $18/hour and the number of hours increased slightly as the amount of feed produced increased. The amount of feed which is produced is not expected to have a large influence on the length of time which the entire process takes because a large portion of the time will be spent setting up and cleaning equipment, rather than operating it.

The overhead costs were estimated to be 18% of the raw material costs.

The distribution costs were estimated to be 10% of the total of the raw materials and overhead costs.

The ex factory price has been calculated per day for each production rate as well as per kilogram of feed produced. Obviously, the ex factory cost will increase as the amount of production increases, but the ex factory cost will decrease substantially as the number of units (or mass of product) increases.

The estimated retail price for the goldfish feed is $120/kg. Most goldfish feeds which are available in pet stores sell for $110/kg to $180/kg. It is recommended that the sustainable goldfish feed is to be priced at the low end of the scale. GST of 12.5% was removed and the retailer's margin of 30% was removed from the retail price of the feed to find the purchase price.

The expected net profit margin was found to range from $19.88 to $39.94 per kilogram, which was between 24.2% and 48.7%.

9.4 Payback period

The payback period is expected to vary substantially with different amounts of product produced (table 10). If fifty kilograms can be produced then the expected payback period is only half a year. This is very unlikely because the surface area which is available of duckweed will be very limiting. Market demand could also be limiting.

Table 10: Expected payback period

Mass of feed produced per day	7	10	20	30	50
Net profit per day ($)	$139.16	$219.38	$654.75	$1,090.13	$1,996.88
Net Profit per year ($)	$34,790.94	$54,844.19	$163,688.39	$272,532.58	$499,220.97
Payback period (years)	7.39	4.69	1.57	0.94	0.51

10.0 Overall Conclusions

- Duckweed can be harvested at a rate of $89 g/m^2/day$, but growth rates will be increased over summer and decreased over winter.
- The surface area of the duckweed pond is severely limiting and it is recommended that more ponds will need to be identified.
- The fish feed meets most of the nutritional requirements of goldfish.
- The required physical properties of the goldfish feed can be met using extrusion.
- It is believed that the fish feed could be very profitable if more duckweed becomes available.
- It is recommended to set the retail price of the feed at $120 per kilogram, which will place it among the cheaper brands on the market.
- The goldfish food will still need to be tested and compared to a current commercial food to determine if it affects the growth rate or the mortality rate of the feed.
- Further research needs to be done into packaging and marketing techniques.

11.0 References

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12.0 Appendix

12.1 Compositions of possible ingredients

12.1.1 Raw carrot nutritional composition

Component	proportion (%)	proportion (% dry matter)
Water	89.9	0
Total protein	0.7	6.93
Total fat	0.4	3.96
Total carbohydrate	5.9	58.42
Fibre	2.9	28.71
Amino Acids		
Arginine	0.022	0.218
Histidine		
Isoleucine	0.02	0.198
Leucine	0.026	0.257
Lysine	0.024	0.238
Methionine	0.006	0.059
Phenylalanine	0.018	0.178
Threonine	0.018	0.178
Tryptophan	0.006	0.059
Valine	0.03	0.297
Essential fatty acids		
Linolenic acid	0.029	0.287
Linoleic acid	0.206	2.040
Minerals		
Calcium	0.0246	0.2436
Magnesium	0.0096	0.0950
Iron	0.00024	0.0024
Copper	0.000036	0.0004
Zinc	0.00019	0.0019
Mangenese	0.00036	0.0036
Phosphorous	0.0331	0.3277
Vitamins		
Thiamin	0.00004	0.0004
Riboflavin	0.000033	0.0003
Pyridoxine	0.000119	0.0012
Panthothenic acid	0.00028	0.0028
Niacin	0.001	0.0099
Biotin	0.0000034	0.0000
Choline		
Inositol		
Vitamin A	0.00979	0.0969
Vitamin E	0.00055	0.0054
Vitamin C	0.00701	0.0694

Adapted from FCDB no. 0065 of Technical University of Denmark

12.1.2 Lettuce waste nutritional composition

Component	proportion (%)	proportion (% dry matter)
Water	95.1	0
Total protein	1.4	28.57
Total fat	0.2	4.08
Total carbohydrate	1.5	30.61
Fibre	1.3	26.53
Amino Acids		
Arginine	0.072	1.469
Histidine		
Isoleucine	0.085	1.735
Leucine	0.078	1.592
Lysine	0.085	1.735
Methionine	0.016	0.327
Phenylalanine	0.055	1.122
Threonine	0.059	1.204
Tryptophan	0.009	0.184
Valine	0.07	1.429
Essential fatty acids		
Linolenic acid	0.056	1.143
Linoleic acid	0.023	0.469
Minerals		
Calcium	0.036	0.7347
Magnesium	0.013	0.2653
Iron	0.00086	0.0176
Copper	0.000029	0.0006
Zinc	0.00018	0.0037
Mangenese	0.00025	0.0051
Phosphorous	0.029	0.5918
Vitamins		
Thiamin	0.00007	0.0014
Riboflavin	0.00008	0.0016
Pyridoxine	0.00009	0.0018
Panthothenic acid	0.000134	0.0027
Niacin	0.000525	0.0107
Biotin		
Choline		
Inositol		
Vitamin A	0.00114	0.0233
Vitamin E		
Vitamin C	0.018	0.3673

Adapted from FCDB no. 0670 of Technical University of Denmark

12.1.3 Duckweed nutritional composition (grown in nutrient rich conditions)

Component	**proportion (% dry matter)**
Water	*≈93% of total mass*
Total protein	41.7
Total fat	4.4
Total carbohydrate	17.6
Fibre	15.6
Amino Acids	
Arginine	2.14
Histidine	0.73
Isoleucine	1.66
Leucine	2.89
Lysine	1.85
Methionine	0.64
Phenylalanine	1.75
Threonine	1.68
Tryptophan	0.40
Valine	2.12
Essential fatty acids	
Linolenic acid	
Linoleic acid	
Minerals	
Calcium	
Magnesium	
Iron	
Copper	
Zinc	
Mangenese	
Phosphorous	
Vitamins	
Thiamin	
Riboflavin	
Pyridoxine	
Panthothenic acid	
Niacin	
Biotin	
Choline	
Inositol	
Vitamin A	
Vitamin E	
Vitamin C	

Adapted from table 3 of Landesman *et al* (n.d.)

12.1.4 Spirulina nutritional composition

Component	proportion (%)	proportion (% dry matter)
Water	5	0
Total protein	60	63.16
Total fat	5	5.26
Total carbohydrate	15	15.79
Fibre	8	8.42
Amino Acids		
Arginine	4.17	4.389
Histidine	0.97	1.021
Isoleucine	3.4	3.579
Leucine	5.24	5.516
Lysine	2.81	2.958
Methionine	1.36	1.432
Phenylalanine	2.72	2.863
Threonine	3.1	3.263
Tryptophan	0.87	0.916
Valine	3.88	4.084
Essential fatty acids		
Linolenic acid	1	1.053
Linoleic acid	0.625	0.658
Minerals		
Calcium	0.7	0.7368
Magnesium	0.4	0.4211
Iron	0.15	0.1579
Copper	0.0012	0.0013
Zinc	0.003	0.0032
Mangenese	0.005	0.0053
Phosphorous	0.8	0.8421
Vitamins		
Thiamin	0.0035	0.0037
Riboflavin	0.004	0.0042
Pyridoxine	0.0008	0.0008
Panthothenic acid	0.0001	0.0001
Niacin	0.014	0.0147
Biotin	0.000005	0.0000
Choline		
Inositol	0.064	0.0674
Vitamin A	0.14	0.1474
Vitamin E	0.01	0.0105
Vitamin C	0	0.0000

Adapted from Life Research Universal (2004) and table 1 of Colla *et al* (2003)

12.1.5 Pea flour composition

Component	proportion (%)	proportion (% dry matter)
Water	9.5	0
Total protein	22	24.31
Total fat	2.1	2.32
Total carbohydrate	63.9	70.61
Fibre	7.4	8.18
Amino Acids		
Arginine	2	2.210
Histidine	0.49	0.541
Isoleucine	0.92	1.017
Leucine	1.4	1.547
Lysine	1.5	1.657
Methionine	0.17	0.188
Phenylalanine	0.92	1.017
Threonine	0.74	0.818
Tryptophan	0.18	0.199
Valine	1.2	1.326
Essential fatty acids		
Linolenic acid	0.197	0.218
Linoleic acid	0.948	1.048
Carbohydrates		
Starch	39.4	43.54
Minerals		
Calcium	0.0378	0.0418
Magnesium	0.082	0.0906
Iron	0.0055	0.0061
Copper	0.00069	0.0008
Zinc	0.0038	0.0042
Mangenese	0.0012	0.0013
Phosphorous	0.407	0.4497
Vitamins		
Thiamin	0.00082	0.0009
Riboflavin	0.00018	0.0002
Pyridoxine	0.000075	0.0001
Panthothenic acid	0.0002	0.0002
Niacin	0.0055	0.0061
Biotin	0.0000005	0.0000
Choline		
Inositol		
Vitamin A	0.00125	0.0014
Vitamin E	0	0.0000
Vitamin C	0.001	0.0011

12.1.6 Corn flour composition

Component	proportion (%)	proportion (% dry matter)
Water	11.1	0
Total protein	6.8	7.65
Total fat	2.8	3.15
Total carbohydrate	75.5	84.93
Fibre	3.2	3.60
Amino Acids		
Arginine	0.28	0.315
Histidine	0.19	0.214
Isoleucine	0.25	0.281
Leucine	0.86	0.967
Lysine	0.19	0.214
Methionine	0.13	0.146
Phenylalanine	0.35	0.394
Threonine	0.25	0.281
Tryptophan	0.044	0.049
Valine	0.33	0.371
Essential fatty acids		
Linolenic acid	0.04	0.045
Linoleic acid	1.14	1.282
Carbohydrates		
Starch	74.2	83.46
Minerals		
Calcium	0.006	0.0067
Magnesium	0.047	0.0529
Iron	0.0011	0.0012
Copper		
Zinc	0.0005	0.0006
Mangenese	0.0000006	0.0000
Phosphorous	0.099	0.1114
Vitamins		
Thiamin	0.00033	0.00037
Riboflavin	0.00011	0.00012
Pyridoxine	0.00033	0.00037
Panthothenic acid		
Niacin	0.00057	0.00064
Biotin		0.00000
Choline		0.00000
Inositol		0.00000
Vitamin A	0.000097	0.00011
Vitamin E	0.00111	0.00125
Vitamin C		0.00000

12.2 Proximate analysis of snow pea waste.

12.2.1 Air over method for moisture determination

#	Tray + Lid	Tray + Lid + Sample	Sample	Dry Tray + Lid + Sample	Dry Sample	Dry Matter
236	25.0890	27.0001	1.9111	25.4255	0.3365	17.608%
140	28.8819	30.7452	1.8633	29.2145	0.3326	17.850%
26	30.7742	33.4495	2.6753	31.2776	0.5034	18.817%
124	26.4495	28.6214	2.1719	26.8612	0.4117	18.956%
26176	26.6129	28.7365	2.1236	27.0180	0.4051	19.076%
						18.461%

Time = 10:30

12.2.2 Soxhlet extraction for lipid determination

10th May

Soxhlet Extraction – Fat

	round bottomed flask	sample	fat + flask	fat	− Blank	% Fat
1	96.9678	10.3103	97.1038	0.1360	0.1330	1.29
2	93.2413	10.0919	93.3711	0.1298	0.1268	1.26
3	101.9657	10.2150	102.1032	0.1375	0.1345	1.35
Blank	101.1579	0	101.1609	0.0030		1.30%

Digestion started at 9:30am, out at ~2pm

12.2.3 Kjeldahl method for protein determination

Kjeldahl – Protein

all have 2 kjeltec tablets + 17 mL H_2SO_4 (× 6)

	sample + boat	boat (~~sample~~)	sample	HCl start	HCl stop	mL HCl	~~% N~~ – Blank	% N	% Protein
1	1.0510	0.4741	0.5769	0.08	13.40	13.32	~~3.232~~ 13.02	3.160	18.96%
2	1.0488	0.4745	0.5743	0.12	13.42	13.30	~~3.242~~ 13.00	3.170	19.02%
3	1.0705	0.4744	0.5961	0.10	14.20	14.10	~~3.335~~ 13.80	3.241	19.45%
Blank	~~1.0703~~		0	0.22	0.52	0.30	0		19.14%

Digestion started at 9:45am HCl = 0.1M

$$\%N = \frac{mL(HCl) \times M(HCl) \times 14 \times 100}{1000 \times M_{(sample)}}$$

COLLINS A4/50 DL NCR

12.2.4 Muffle furnace method for ash determination

Ash Determination

#	crucible	crucible + sample (~~ash~~)	sample	crucible + ash	ash	ash %
~~2~~	~~14.8148~~					
34	21.4079	23.3378	1.9299	21.4627	0.0548	2.84%
14	18.5701	20.5278	1.9577	18.6275	0.0574	2.93%
21	22.5412	24.4648	1.9236	22.5961	0.0549	2.85%
						2.87%

into muffle furnace at 10:25am, out at ~3pm

12.3 Fertiliser composition

Fertilizer		g/1000 L =1ppm	Fert. In Kg	PPM in feed
Calcium Nitrate	N	6.45	18.7	96.64
	Ca	4.7	18.7	132.62
Potassium Nitrate	N	7.3	10.55	48.17
	K	2.7	10.55	130.25
Magnesium Sulphate	Mg	10.75	9.5	29.46
	SO4	7.69	9.5	41.18
MKP	K	3.53	4.22	39.85
	P	4.45	4.22	31.61
Potassium Sulphate	K	2.5	5.275	70.33
	SO4	5.56	5.275	31.62
Zinc Sulphate	Zn	4.42	0.021	0.16
Manganese Sulphate	Mn	4.05	0.094	0.77
Iron Chelate 9%	Fe	11.1	0.6	1.80
Iron Sulphate	Fe	5.54		0.00
Copper Sulphate	Cu	3.91	0.012	0.10
Ammonium Nitrate	NH4	6.02	0.79	4.37
	NO3	6.02	0.79	4.37
Boric Acid	B	5.64	0.047	0.28
Sodium Molvbdate	Mo	2.56	0.002	0.03
Tank and Injector		tank size in L	Injector xxx:1	
		200	150	30

Crop			Tomato pumwh			
N	149.19	ppm	Tank A			
			CaNO3	18.70	Kg.	7.508
P	31.61	ppm	KNO3	2.11	Kg.	0.847
			Fe Chelate 9%	0.60	Kg.	0.241
K	240.43	ppm	NH4NO3	0.79	Kg.	0.317
				22.20		8.914
Ca	132.62	ppm	Tank B			
SO4	72.80	ppm	KNO3	8.44	Kg.	3.389
			KSO4	5.28	Kg.	2.118
Mg	29.46	ppm	MKP	4.22	Kg.	1.694
			MgSO4	9.50	Kg.	3.814
Fe	1.80	ppm		27.44		11.016
Mn	0.77	ppm	MnSO4	94.00	grams	37.743
			ZnSO4	21.00	grams	8.432
Zn	0.16	ppm	CuSO4	12.00	grams	4.818
			Boric Acid	47.00	grams	18.871
Cu	0.10	ppm	NaMo	2.00	grams	0.803
				0.18		0.071
NH4	4.37	ppm				
						0.401518
B	0.28	ppm		49.81		20.00
			Tank and Injector			
Mo	0.03	ppm	tank size in L	Injector xxx:1		
			200	150	30	

12.4 Log of changes made to duckweed tanks.

Glasshouse # 4 - ADRIAN HOGGARD.

Name	Date	Activity / Consumables
	13/6/09	weed into tanks 1-4
	22/6/09	Top up all tanks 2½ l 10 30am
		60mls A 60mls B into 5l tap water for topups
		25,25 25,28, 25,30
		1 drop in 2.5 l no harm to pond weed but algae still
		1 drop in 2.0 l
	21/7/09	Dosed tank 5 with 28 drops
	22/7/09	Dosed all remaining tanks 28 drops
	28-7-09	Dose tanks again
	30-7-09	Top up tanks 5 l 2 drops
	31/7	Harvest
	3/8	Top up tanks 5l 3 drops
	3/8	Harvest
	5/8	Harvest
	10/8	Top up tanks 10 l in each 3drops in 5 l + top up nutrients 60 A 60 B in 5 l
	11/8	5 l into each top up (with 30 drops of algae killer but no nutrients)
	12/8	Harvest
	12/8	5 l into each top up (" " ") first t[illegible]
	13/8	Spray tops of tanks.
	17/8	Harvest
	17/8	Top up tanks 10 l in each (30 drops alga killer into [illegible] + spray tops no nutrie[illegible]
	18/8	aphids on the weed. ~~fly w~~ spray with fly spray
	19/8	Harvest
	19/8	Top up tanks 5 l and spray across tops
	21/8	Harvest
	24/8	Harvest
	"	Top up tanks 15 l + algae killer into each tank
	25/8	Spray accross tops
	26/8	Harvest
	31/8	Top up tanks 10L ~~with~~ + Algae killer 60 drops into each + spray over top
	1/9	Harvest
	3/9	Top up tanks 10L + Algae killer + 100mls A+B solution [illegible]
	4/9	Top up tanks H_2O only Algae killer sprayed
	7/9	Harvest

drops 6/9 Top up 15Lt EA

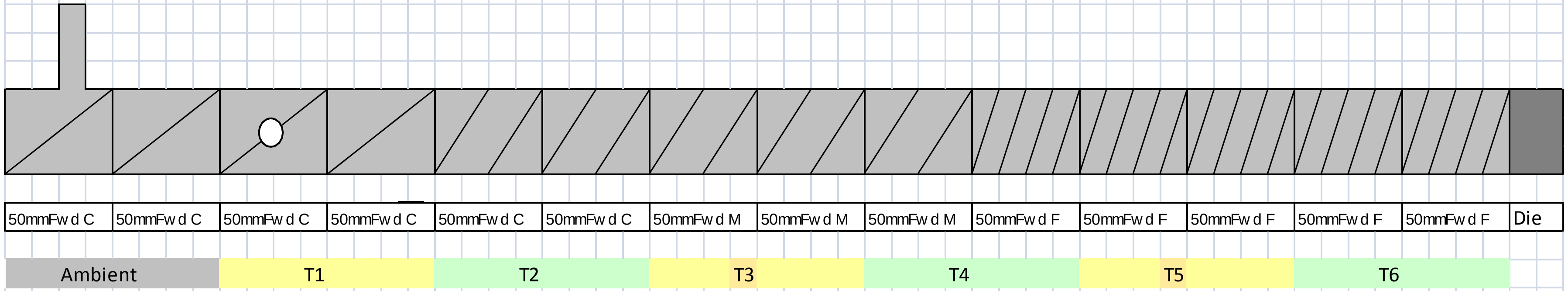
12.5 Log of duckweed drying

29/07/09

Date	kg Fresh	kg Dry	weight loss / ~~Moisture~~	Time in / Temp	Time out.	
29/07/09	2.695 + 2.240 -3.025 = 1.46kg	0.105 kg	94.6%	10:30am / 60°C	4:30pm 30/07	trays = 3.025kg
31/07/09	3.170 + 3.030 - 4.785 = 1.415kg	0.080 kg	94.3%	10:30am / 70°C	~~11:30~~ 12:30pm 01/08	trays = 4.785 kg
03/08/09	3.150 + 3.340 -4.785 = 1.705	0.085 kg	95.0%	11:30am / 60°C	3:30 pm 04/08	trays = 4.785kg
05/08/09	3.225 + 3.200 -4.785 = 1.640kg	0.100 kg	93.9%	11:00 am / 59°C	3:30pm 06/08	trays = 4.785k
07/08/09	3.185 + 3.170 -4.785 = 1.570kg	0.080 kg	94.9%	10:00am / 60°C	3:00pm 09/08	trays = 4.785 kg
10/08/09	3.950 - 2.390 = 1.56 kg	0.065 kg	95.8%	10:00am / 70°C	3:30pm 11/08	tray = 2.390kg
12/08/09	4.000 - 2.390 = 1.61 kg	0.065 kg	96.0%	11:00am / 66°C	4:00pm 13/08	tray = 2.390 kg
17/08/09	3.500 + 3.390 - 4.810 = 2.08	0.085 kg	95.9%	11:00am / 60°C	3:00pm 18/08	trays = 4.810kg
19/08/09	3.955 + 2.405 -4.805 = 1.555	0.075kg	95.2%	11:00am / 70°C	4:00pm 20/08	trays = 4.805 kg
21/08/09	3.795 - 2.390 = 1.405	0.065kg	95.4%	9:00am / 70°C		tray = 2.390 kg
24/08/09	3.200 - 1.455 = 1.745	0.090kg	94.8%	1:00pm / 70°C	3:30pm 25/08	tray = 1.455 kg
26/08/09	3.030 - 1.455 = 1.575	0.070kg	95.6%	10:00am / 80°C	2:00pm 27/08	tray = 1.455kg
1/09/09	3.450 + 3.355 - 4.790 = 2.045	0.100 kg	95.8%	11:00am / 70°C		trays = 4.790k
7/09/09	3.360 + 3.340 - 4.790 = 1.91	0.125kg	93.5%			trays = 4.790 kg
09/09/09	3.095 + 3.625 -4.790 = 1.93	0.090kg				
		Average:	95.1%			
16/09/09	4.140 + 4.075 + 4.230 + 4.360 - 9.580 = 7.225	0.435kg				trays = 9.580
18/09/09	4.580 + 4.840 + 4.72 + 4.44 - 9.580 = 9.00					

collins A4/50 DL NCR

12.6 Extruder conditions used during trials



	Run 1 25/08/2008			Run 2 18/09/2009		
	A	B	C	A	B	C
T1 (°C)	50	50	50	50	50	50
T2 (°C)	90	90	90	90	90	90
T3 (°C)	126	126	115	118	119	119
T4 (°C)	119	119	122	118	120	120
T5 (°C)	60	60	91	60	119	119
T6 (°C)	51	51	89	53	101	101
D1 (Torque)	6	6	5	8	3.5	5.5
D2 (Screw Speed)	99	99	96	99	99	99
D3 (Feed Rate)	35	35	35	69	70	80
D4 (Water Flow Rate)	.37x½	.37x¼	.37x¼	.39x½	.6x½	.6x½
D5 (Blade Speed)	196	197	197	189	186	190
Pressure, Bars	110	110	75	140	45	55

NOTE: Highlighted blue cells are those which had been changed since the previous set of conditions

12.7 Floating and sinking raw data

Sample A - Nutrafin		
Floating (s)	Sinking (s)	Sinking Time
0	5.15	5.15
12.34	21.78	9.44
1.31	6.45	5.14
120		
1	10.31	9.31
0	5.11	5.11
1.3	7.19	5.89
1.95	8.11	6.16
120		
1.75	8.84	7.09
3.78	15.95	12.17
0	6.17	6.17
2.47	8.78	6.31
120		
120		
3.3	13.27	9.97
2.4	10.15	7.75
13.81	44.28	30.47
2.33	11.12	8.79
120		
6.84	11.84	5
120		
2.63	6.44	3.81
120		
120		
1.94	6.34	4.4
15.15	53.69	38.54
1.15	6.42	5.27
1.49	11.95	10.46
120		

Sample B - TetraColor		
Floating (s)	Sinking (s)	Sinking Time
8.84	16.52	7.68
3.2	9.5	6.3
3.98	11.86	7.88
1.89	8.98	7.09
6.28	14.51	8.23
0	8.31	8.31
1.64	9.67	8.03
1.59	8.59	7
0	7.78	7.78
2.81	9.61	6.8
0	8.44	8.44
0.88	7.88	7
0	7.69	7.69
3.8	11.24	7.44
0	9.71	9.71
1.17	8.61	7.44
0	8.53	8.53
2.66	12.72	10.06
0	9.5	9.5
0	8.88	8.88
9.81	18.75	8.94
5.31	13.28	7.97
1.31	8	6.69
0	8.27	8.27
1.19	8.72	7.53
7.8	15.92	8.12
0	7.59	7.59
33.61	39.58	5.97
0	7.39	7.39
2.28	10.56	8.28

1 - A w/o Oil		
Floating (s)	Sinking (s)	Sinking Time
0	3.17	3.17
0	3.3	3.3
0	3.41	3.41
0	4.22	4.22
0	3.83	3.83
0	3.6	3.6
0	3.27	3.27
0	3.47	3.47
0	4.42	4.42
0	3.38	3.38
0	3.39	3.39
0	4.11	4.11
0	3.74	3.74
0	4.94	4.94
0	3.44	3.44
0	4	4
0	3.58	3.58
0	4.3	4.3
0	3.95	3.95
0	3.94	3.94

2 - A w Oil		
Floating (s)	Sinking (s)	Sinking Time
0	3.63	3.63
0	3.44	3.44
0	3.59	3.59
0	3.92	3.92
0	3.72	3.72
0	4.34	4.34
0	3.59	3.59
0	3.36	3.36
0	3.53	3.53
0	3.67	3.67
0	3.44	3.44
0	3.45	3.45
0	3.61	3.61
0	3.5	3.5
0	3.52	3.52
0	3.47	3.47
0	4.22	4.22
0	3.6	3.6
0	3.58	3.58
0	3.23	3.23

3 - B w/o Oil		
Floating (s)	Sinking (s)	Sinking Time
0	4.44	4.44
0	4.24	4.24
0	4.91	4.91
0	4.89	4.89
0	4.38	4.38
0	4.47	4.47
0	3.99	3.99
0	4.67	4.67
0	4.3	4.3
0	4.77	4.77
0	4.5	4.5
0	4.38	4.38
0	5.82	5.82
0	4.81	4.81
0	4.97	4.97
0	4.17	4.17
0	4.16	4.16
0	4.16	4.16
0	4.86	4.86
0	4.19	4.19

4 - B w Oil		
Floating (s)	Sinking (s)	Sinking Time
0	5.47	5.47
0	4.56	4.56
0	4.61	4.61
0	4.56	4.56
0	5.17	5.17
0	3.94	3.94
0	3.78	3.78
0	5.19	5.19
0	4.72	4.72
0	3.81	3.81
0	5.78	5.78
0	3.86	3.86
0	5.25	5.25
0	4.62	4.62
0	4.33	4.33
0	4.16	4.16
0	4.42	4.42
0	3.74	3.74
0	4.44	4.44
0	4.31	4.31

5 - C w/o Oil		
Floating (s)	Sinking (s)	Sinking Time
120		
0	7.35	7.35
120		
120		
70	107	37
120		
120		
120		
120		
120		
0	7.16	7.16
120		
120		
5.42	23.42	18
120		
0	4.41	4.41
0	10.28	10.28
0	6.89	6.89
120		
120		

6 - D w Oil		
Floating (s)	Sinking (s)	Sinking Time
120		
2.11	13.48	11.37
1.69	10.93	9.24
120		
120		
18.7	44.9	26.2
120		
120		
0	13.67	13.67
0	7.69	7.69
120		
120		
120		
120		
2.61	16.55	13.94
1.94	13.55	11.61
120		
120		
120		
4.2	18.8	14.6

12.8 Sieving data

TOTAL MASSES					
Sieve Size	Mass(g)	Commercial A (g)	Commercial B (g)	Sample 3 (g)	Sample 5 (g)
850µm	322.94	423.06	415.39	422.79	422.38
600µm	325.46	325.46	330.89	325.53	325.57
355µm	301.36	301.36	303.35	301.43	301.54
212µm	281.3	281.3	281.63	281.39	281.41
0µm	485.03	485.03	485.34	485.12	485.16

PARTICLE SIZE DISTRIBUTION (Mass)					
Sieve Size	Mass(g)	Commercial A (g)	Commercial B (g)	Sample 3 (g)	Sample 5 (g)
850µm	322.94	100.12	92.45	99.85	99.44
600µm	325.46	0	5.43	0.07	0.11
355µm	301.36	0	1.99	0.07	0.18
212µm	281.3	0	0.33	0.09	0.11
0µm	485.03	0	0.31	0.09	0.13
	SUM	100.12	100.51	100.17	99.97

PARTICAL SIZE DISTRIBUTION (%)					
Sieve Size	Mass(g)	Commercial A (g)	Commercial B (g)	Sample 3 (g)	Sample 5 (g)
850µm	322.94	100.0	92.0	99.7	99.5
600µm	325.46	0.0	5.4	0.1	0.1
355µm	301.36	0.0	2.0	0.1	0.2
212µm	281.3	0.0	0.3	0.1	0.1
0µm	485.03	0.0	0.3	0.1	0.1
	SUM	100	100	100	100

12.9 Observations made during hardness testing

28/09/09

Texture analysis

Nutrafin 01	very obvius cracking	5Sample 01	cracking, quite low pitch ↑
Nutrafin 02	" "	5Sample 02	" " hard gr
Nutrafin 03	"	5 Sample 03	" " "
Nutrafin 121	slight crack noise	5 sample121	very quiet crack, 1 small grit
Nutrafin 122	soft crack noise, (hard) centre	5 sample122	no audible crack, small grit
Nutrafin123	soft crack noise with (hard) grit in centre	5 sample 123	very quiet crack, slightly larger grit
Nutrafin301	quiet crack with hard grits	5 sample301	no noise, soft almost right thro
Nutrafin 302	soft crushing noise, (lots of cracks) grits	5 sample302	very slight crushing noise, (soft) mostly
Nutrafin 303	very little noise still grits	5 sample303	1 very quiet crack, "
Tetracolor 01	cracking, not very sharp noise	3 sample 01	two quiet cracks
Tetracolor 02	lots of cracking	3 sample 02	loud obvious crack, then another, (hard) fragm
Tetracolor03	two high pitched cracks	3 sample 03	quite loud crack, hard fragments
Tetracolor121	no cracking noise, soft (whole way thro)	3 sample121	(reasonably) obvious crack, some hard grits
Tetracolor122	" "	3 sample122	quiet crack, hard grits. but rest s
Tetracolor123	" "	3 sample123	" " "
Tetracolor301	no noise, soft whole way through	3 sample301	1 very faint crack, small gr
Tetracolor302	" " "	3 sample302	1 soft crack noise, 2 hard grits
Tetracolor303	" " "	3 sample303	very quiet cracking, some dig bi