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Swedish University of Agricultural Sciences
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Dairy waste – Feed for fish?

Thomas Eliasson

Examensarbete / SLU, Institutionen för husdjurens utfodring och vård, **531**

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Mejeriavfall – Foder till fisk?

Thomas Eliasson

Handledare: Su-Lin Hedén, SLU, Inst. för mikrobiologi
Supervisor:
Examinator: Torbjörn Lund, SLU, Inst. för husdjurens utfodring och vård
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Abstract

Global meat production has increased dramatically in the last 50 years and along with this growth, aquaculture has made a rapid development, now comprising 14.3 % of all meat produced. The growing demand and desire for animal protein will become a real challenge in the future, seafood derived from aquaculture may be a partial solution, showing positive effects on both undernourishment and obesity in humans. But as with other types of animal production, the most demanding challenge is feed supply, there is always the risk of feed ingredients competing with foods for human consumption. In 2008, from major groups of fish and crustaceans cultured, 64.4 % of the production was dependent on external feeds, of which ingredients based on fish and other water-living animals comprised 15.4 %. Further investigations showed that there are major differences in feed consumption (both type of feed and amount) between species due to a wide diversity within their digestive systems and nutrient requirements. Single cell protein (SCP) is a feed source with major potential for aquaculture, supported by the fact that fish and crustaceans possesses the ability to degrade nucleic acids (purines) more efficiently than other animals. A short review based on 10 earlier studies where fish meal was substituted by yeast SCP showed mixed positive and negative results on growth; more research is needed. A goal of this study was to produce SCP from dairy wastewater by fermentation with the yeast *Kluyveromyces lactis*, and in the process also improve the wastewater for biogas production. The results showed that there may be a beneficial effect for biogas production, whereas the SCP production was less promising. The biomass increased by 57.84 % (after 30 hours of fermentation), which is positive. Though, further analysis of the pelleted biomass indicated a decreasing concentration of crude protein per kg dry matter.

Sammanfattning

De sista 50 åren har den globala köttproduktionen ökat väldigt kraftigt. I anslutning till denna utveckling har aquakultur visat en mycket positiv tillväxt, idag utgör aquakultur 14,3% av allt kött producerat. Förutom ett ökat behov finns också en ökad efterfrågan på animaliskt protein vilket kommer bli en stor utmaning i framtiden. Produktion av livsmedel från aquakultur kan vara en del av lösningen på problemet då tydliga positiva hälsoeffekter visats, både när det gäller undernäring och övervikt. Men precis som med andra typer av djurproduktion utgör också fodret här en stor utmaning, det finns alltid en risk för konkurrens med human konsumtion. Tittar man på de mest odlade arterna av fisk och skaldjur 2008, var 64,4% av produktionen beroende av inköpt eller producerat foder. Fisk och andra vattenlevande organismer utgjorde då 15,4% av ingredienserna till dessa foder. Vidare undersökningar visade på stora skillnader i foderkonsumtion mellan de odlade arterna, både vad gäller typ och mängd av foder. Dessa skillnader visade sig bero på variationer inom både digestionsfysiologi och näringsbehov. Mikrobiellt protein utgör en foderkälla med stor potential inom aquakultur, detta baserat på att fisk och skaldjur bryter ned nukleinsyror (puriner) effektivare än andra djur. En kort genomgång av 10 tidigare studier där man testat att byta ut fiskmjöl mot mikrobiellt protein, visar både positiva och negativa effekter på tillväxt; mer forskning behövs dock. Ett av målen med denna studie var att undersöka möjligheten att producera mikrobiellt protein på mejeriavfall genom fermentering, med hjälp av jästarten *Kluyveromyces lactis*. Förutom att producera mikrobiellt protein, undersöks också om mejeriavfallet förbättrats med avseende på biogasproduktion. Resultaten tyder på att det kan vara en fördel för biogasutbytet medan produktionen av mikrobiellt protein verkar mindre lovande. Fermenteringen gav en ökning i biomassa på 57,84% (efter 30 timmar) vilket är positivt, vidare analyser av den pelleterade biomassan tyder dock på en minskad koncentration av råprotein per kg torrvikt.

1 Introduction

One of the most demanding questions for mankind now and in the future, is how to secure a safe and sustainable supply of food for a growing world population. In the beginning of this century, United Nations (UN) declared the Millennium Development Goals (MDG) with a deadline set for 2015. At least three of these goals were directly coupled to food security, until now much has been done but still more is needed to eradicate hunger and undernourishment (United Nations, 2014). The latest report from FAO *et al.* (2015) gives us the hard facts, more than 1/10 of the world's population still suffers from undernourishment in some way.

1.1 Protein of animal origin

The growing desire and need for protein of animal origin is a real challenge for our planet that is difficult to preempt. Livestock production uses much water and land that instead could be used directly by humans, and also contributes substantially to greenhouse gases, having a large impact on climate change (Steinfeld *et al.*, 2006).

The fish industry on the other hand suffers from depleted fish populations and increasingly stringent regulations to compensate for long periods of overfishing. Today almost 80 % of the global fish supplies are overfished or fished to their maximum level. This negative development has been accompanied by the rapid growth in aquaculture, which used and still uses fish as feed for fish. Since 2005, there has been a constant rise in price for feedstuffs based on fish, pushing aquaculture to find new feeds (FAO, 2014).

1.1.1 Meat production

Patterns in the global production of meat are changing rapidly. In 1962, almost all meat came from livestock production and caught seafood. Fifty years later, aquaculture has expanded to form a greater proportion of global production. This increase from 1.3 % to 14.3 % within a half century is shown in more detail in figure 1. Meat production in total quadrupled from around 120 to 460 million tons in the same period.

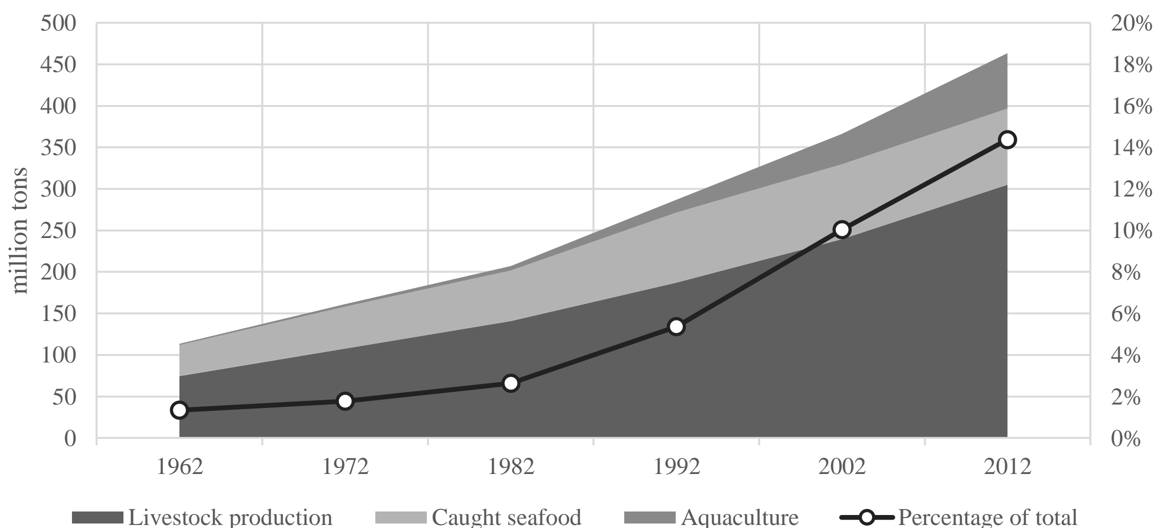

Figure 1 – Total global meat supply. Livestock production is based on data from FAO (2015) representing total production, excluding caught seafood and aquaculture. Aquaculture and Caught seafood is based on data from FAO (2013) representing fish, crustaceans and mollusks. Percentage of total shows the proportion of the total supply accounted for by aquaculture.

Further analysis on the data provided by FAO (2015) shows that meat from aquaculture has surpassed beef, which also highlights the impressive development.

1.2 Seafood and human nutrition

Although there are many drivers for the rapid growth in aquaculture, what is clear is that seafood constitutes a good food source for humans. Aquatic organisms, both plants and animals, seem to have a nutrient profile that is particularly suited to human needs, especially with regard to essential fatty acids, vitamins and minerals. Furthermore, fish in combination with vegetables is recommended to replace red meat as a protein source (Nordic Council of Ministers, 2014).

An important, albeit generalized, trend in human nutrition is that certain people eat both too much and in the wrong proportions, whereas others suffer from undernourishment and a scarce food supply (FAO *et al.*, 2015). These are two contrasting challenges in global food supply and consumption.

1.2.1 Abundance

People with a high living standard tend to consume excessive amounts of red meat, causing obesity and other health problems. Shifting this intake from livestock to seafood may be one part of the solution, with Japan being a good example. Very low rates of obesity and health problems related to nutrition are observed despite the good living standard, a greater proportion of seafood in the Japanese diet seems to be the key (Tacon & Metian, 2013). Health benefits from seafood, especially fish, are also pointed out by the Nordic Council of Ministers (2014).

1.2.2 Scarcity

But there are also those who do not have the opportunity to choose their food. As mentioned earlier, more than 1/10 of the world population suffers from undernourishment, and there is no simple solution to reduce that, but seafood, and especially fish, may be one part of the solution. Deficiency in essential vitamins and minerals, also called hidden hunger, affects many people in developing countries. The most vulnerable groups are children and breastfeeding or pregnant women. Fish from fishing and aquaculture can supply these nutrients. People depending on vegetables as a protein source also often lack the amino acid lysine, which is abundant in most fish. Seafood production, both fishing and aquaculture, contributes to livelihoods, creating new jobs and other economic opportunities in developing countries (Allison, 2011).

1.3 Aquaculture as a future seafood supply

It is clear that there is both a growing need and demand for seafood, and it appears that aquaculture will continue to play an increasing role in this development. But there are many challenges ahead to overcome. The most important is feed supply, currently, fish and soy are the most crucial ingredients in feeds designated for aquaculture (FAO, 2014). We need to find more sustainable ways to produce feeds, using ingredients not competing with human consumption.

1.3.1 Microbial protein

Domestication of microorganisms has led us to new possibilities. Today there is a diversity of products produced directly or with help from microbes, from feed ingredients to metabolites of medical importance. A second observation is that, food and other industries produce much nutrient-rich wastewater (Valta *et al.*, 2015) that is typically treated at the sewer facility, where it is contaminated by other waste flows.

For example, wastewater from the Swedish dairy industry mostly comprises whey but also relatively large proportions of milk and product residues. This may be a good substrate to support the growth of yeasts, based on the relatively high contents of sugars, lipids, proteins and minerals (Waites, 2001).

2 Aims

This study examines the potential to use yeast to produce biomass from dairy wastewater, and also characterizes this biomass to judge its suitability as a protein ingredient in fish feed.

The study is divided into two parts: a literature study and a small laboratory pilot-trial.

The first part consists of a literature study on current trends in aquaculture and the feeds used are presented, followed by a brief description of fish digestive anatomy and nutritional requirements of both fish and crustaceans. Relevant studies of feeding yeast-derived microbial protein to fish are also compared.

The second part presents experimental data obtained by growing yeast on a dairy waste substrate in small scale bioreactors, with a focus on the amount and characteristics of the biomass produced. An additional aspect is that some dairy industries use their wastewater as a substrate for biogas production, often with very variable results. Therefore, the potential impact of yeast treatment on biogas production is also mentioned.

3 Literature study

3.1 Trends in Aquaculture

Feed supply is one of the most crucial problems in attaining sustainable aquaculture production. Based on data from FishStatJ (FAO, 2013), total aquaculture production of fish, crustaceans and mollusks reached 52.3 million tons in 2008. From this production, 11 major groups of fish and crustaceans were identified by Tacon *et al.* (2011) as highly dependent on external nutrients. Further investigation recognized differences in feed dependencies within those groups; proportions of intensive (high input) and extensive (low input) production are presented in figure 2.

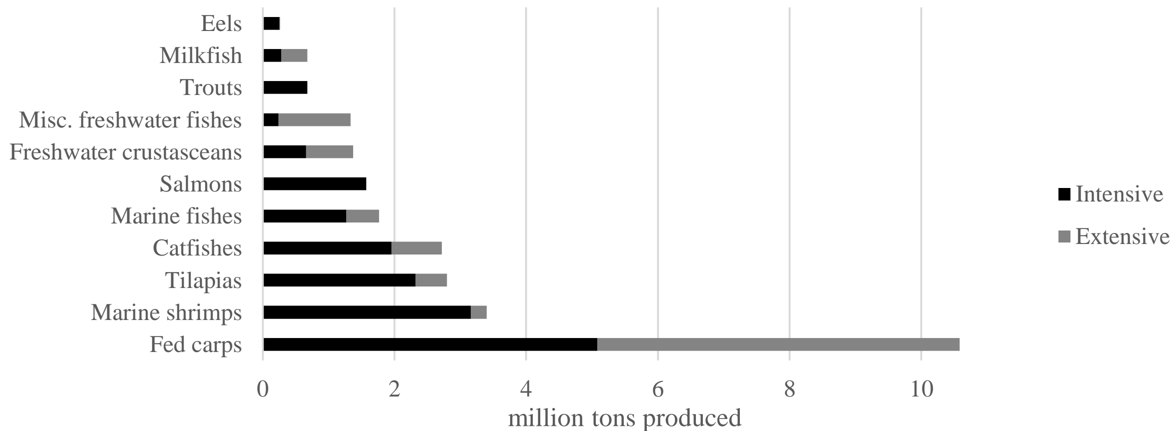

Figure 2 – Feed dependency in major groups of aquaculture 2008, adapted from Tacon *et al.* (2011).

These data indicate that 27.16 million tons was produced by these major groups in 2008, which is 51.9 % of total aquaculture production. Intensive production constituted 17.48 million tons of that amount, which is 64.4 %. Thus, two thirds of the production from these major groups is dependent on external feed sources.

3.1.1 Feed consumption

The total amount of commercial feed consumed in 2008 by the intensive proportion of the production groups (as listed above) is shown in figure 3, also noting the proportions of fish based feed ingredients.

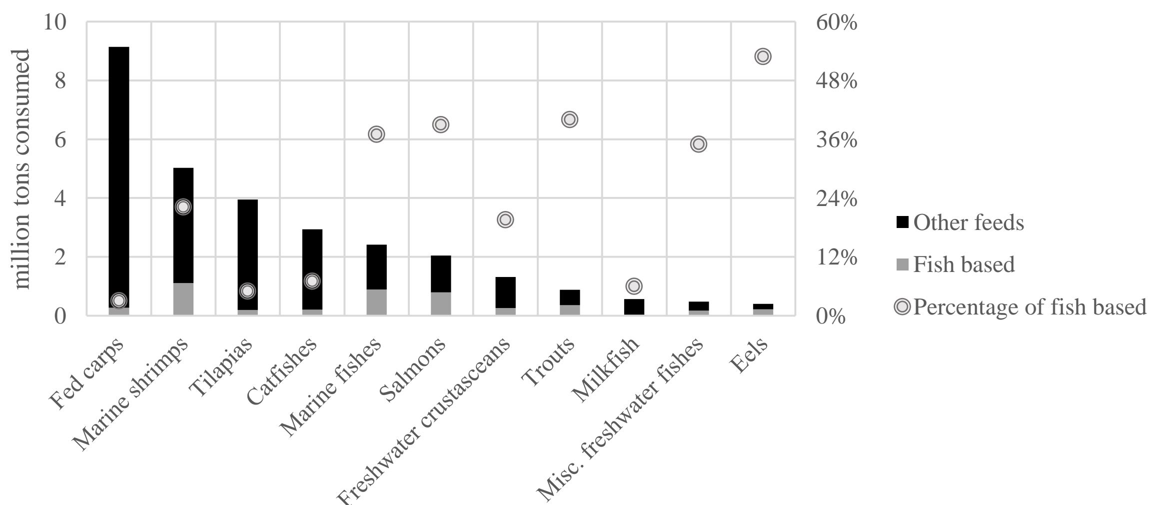

Figure 3 – Consumption of commercial feed by the major groups of aquaculture 2008, adapted from Tacon *et al.* (2011).

Together, these major groups consumed 29.16 million tons of feed, of which fish based feed ingredients comprised 4.50 million tons. These data better highlight the feed supply dilemma, to produce seafood in aquaculture, major amounts of feed are needed. Many of these feed ingredients compete directly with human consumption. The consumption of soy is not presented in detail, however, also important to highlight because of deforestation of rainforests and other environmental concerns.

In the public debate, fish as feed for fish has featured as a major question. Data in figure 3 indicate that feed ingredients based on fish and other water-living animals comprise 15.4 % of total feed consumed. Further analysis also indicates major diversities in consumption between the groups. This is not an alarming proportion, but with the knowledge of global fish supplies depleting (FAO, 2014), major efforts should be directed to find alternatives for fish based feed.

3.1.2 Fish meal and fish oil

Driven by the rapid development in aquaculture, usage of fish meal (FM) and fish oil (FO) as feed ingredients has steadily increased. In 2008, global fisheries and aquaculture production of fish, crustaceans and mollusks reached 143.1 million tons. Processing resulted in 50.9 million tons of commodities where FM and FO constituted 5.26 (10.3 %) respectively 1.07 (2.1 %) million tons of the total amount (FAO, 2013).

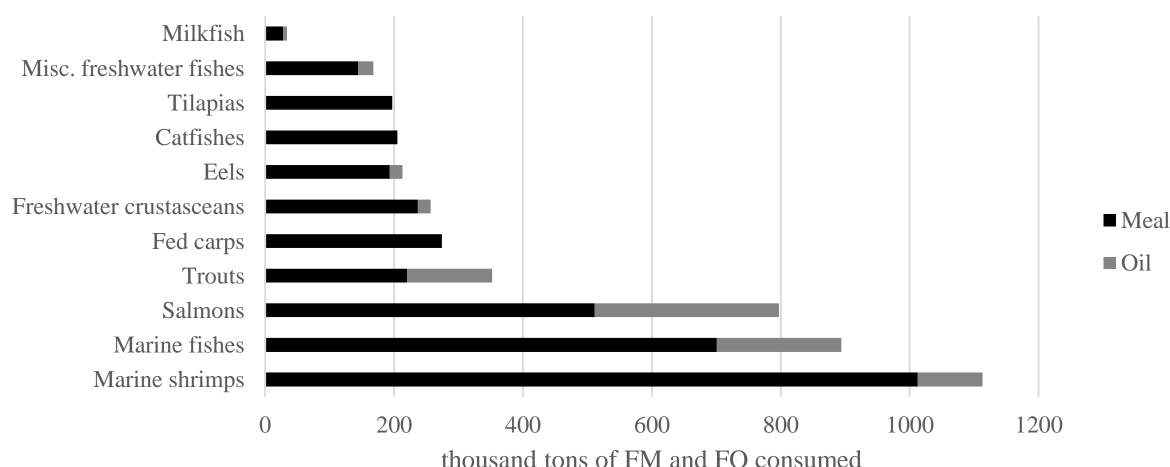

Figure 4 – Consumption of fish based feed ingredients by the major groups of aquaculture 2008, adapted from Tacon *et al.* (2011).

From the total FM and FO produced, the major groups of aquaculture consumed 3.72 (70.7 %) respectively 0.78 (72.9 %) million tons (Tacon *et al.*, 2011). In figure 4, a more detailed view on the consumption of FM and FO is presented.

The utilization of FM and FO shows great diversity between the groups. Surprisingly shrimps turned out to be the top consumer, followed by marine fishes and salmons. It would be strategic to focus on the nutritional requirements and preferences of these “top consumer” groups when finding new ways to meet the need for sustainable feed supplies in aquaculture. However, the most sustainable option might be to not produce these groups at all.

3.1.3 Other feed ingredients

In addition to FM and FO, it is important not to forget that considerable amounts of non-fish based feed ingredients are consumed, 84.6 % of the total 29.16 million tons mentioned earlier. It is difficult to present an exact proportion of each ingredient because of the major variations between species. Hence a rough prediction can be made on the most commonly used protein-rich ingredients, based on different studies summarized by Tacon *et al.* (2009) presented below.

3.1.3.1 Livestock products

Based on different kinds of animals a broad diversity of blood, meat, intestinal and bone meals are produced. Overall mean inclusion level for each ingredient is 2-10 % of total feed, with exception for poultry-by-product-meal that may be included up to 25 % of total feed.

3.1.3.2 Vegetable products

Many protein-rich feed ingredients are also based on vegetable sources, these can be divided into different sub-groups. Products based on cereals comprises brewers grains and gluten meals (especially maize and wheat), mean inclusion levels of each ingredient varies from 3-15 % of total feed. Oilseed products comprise a wide variety of ingredients, based on byproducts from extraction of lipids, mean inclusion levels for each ingredient varies from 3-18 % of total feed. Legume products are derived from soybeans, peas and lupines, mean levels of inclusion for each of these ingredients are 4-20 % of total feed. Many of the oilseeds and legumes, for example the soybean, contains anti-nutritional substances such as lectins, trypsin inhibitors and phytic acid, substances that interfere with nutrient uptake and also may compromise health in the long run (Liener, 1994).

3.1.3.3 Microbial products

Single cell proteins (SCP) based on bacteria and yeast are used in relatively small amounts, mean inclusion level of each ingredient is 1-4 %. This indicates that there may be future possibilities to increase the amount of ingredients of microbial origin.

3.2 Fish and shrimp nutrition

In order to substitute current aquaculture feed ingredients with more sustainable alternatives, a better understanding of fish and shrimp nutritional requirements is needed. The most pressing concern is finding suitable replacements or partial substitutes for FM and FO, but alternatives for other feed components (especially the soybean) that could otherwise be used for human consumption are also of interest.

Among fish species used in aquaculture, the feed preferences vary, from hunting carnivores and planktivores to grass-fed herbivores, with omnivores relying on different feed sources. Aquaculture species also include detritivores (also called scavengers) feeding on dead materials (De Silva and Anderson, 1995; NRC, 2011).

3.2.1 Fish digestive system

The diversity in feed preference originates from evolution of species, where food source and supply has optimally adapted fish to their living environment. Variation in the anatomical structure and function of their digestive systems is a product of this long adaptation and selection. In figure 5 a brief comparison is presented to highlight the broad diversity between different species, also discussed more thoroughly below.

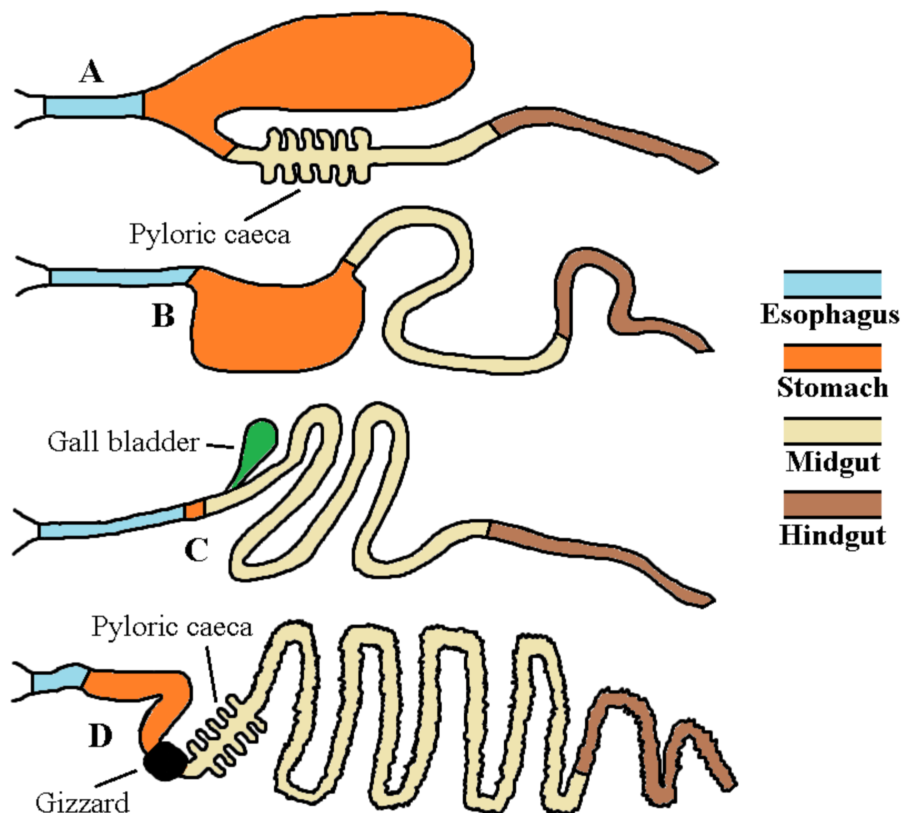

Figure 5 – Comparative digestive anatomy of fish, own interpretation (NRC, 2011). The figure visualizes basic compartments in the digestive system, based on different food preferences. **A:** Carnivore. **B & C:** Omnivores with different strategies. **D:** Microphagous planktivore.

From the groups of species mentioned earlier in section 3.1, some can be categorized into the schematics shown in figure 5. Salmons, trouts and some of the marine fishes belong to A. Catfishes and tilapias belong to B. Most of the fed carps belong to C. Milkfish belong to D (Halver & Hardy, 2002).

3.2.1.1 Mouth and stomach

Ingestion is carried out differently depending on feed preference. Carnivore fish with teeth often catch their pray and tear it into pieces before intake, whereas some species instead suck in their prey together with some water. Herbivores snap off plants and grind them between their teeth. Filter feeders use their gill rakers to catch plankton from large volumes of water passing through the mouth (NRC, 2011).

Most carnivores (and omnivores) which rely on living prey have a distinct stomach, but both shape and size show great diversity as seen in figure 5 (A and B). Herbivores and detritivores often lack a distinct stomach (figure 5, C), and this is as mentioned earlier, the case for most carp-like fishes (De Silva & Anderson, 1995). In milkfish, the stomach has a more tubular-like shape, followed by a gizzard (figure 5, D). The gizzard appear to have similar function as in avian species, apart from secretion which seem to vary between fish species (Ferraris *et al.*, 1987).

These understandings of mouth and stomach physiology highlight the fact that not only nutritional value but also digestible value play a major role when developing new feeds.

3.2.1.2 Intestines

There are major differences between fish and mammals when it comes to nutrient uptake. In fish, nutrients can be absorbed even in the distal parts of the intestine, though, there seem to be variation between species (Ferraris & Ahearn, 1984). Absorption is affected by different factors, and one of the most critical is total surface area. Nutrients in the lumen contents must come into direct contact with epithelial cells, otherwise they will not be absorbed. Surface area can be expanded by increased intestine length, or by formation of bulges as in the pyloric caeca, seen in figure 5 (NRC, 2011). The pyloric caeca found in fish does not function as the caeca in mammals, there is no fermentation or storing capabilities but on the other hand, enzymatic activity and uptake of nutrients is comparable and often also better than in the rest of the proximal intestine. In trouts, the pyloric caeca comprises over 70 % of total intestine surface area (Buddington & Diamond, 1986).

3.2.2 Nucleic acid metabolism

Including high levels of SCP in feed or food might be hazardous, microorganisms contains high amounts of nucleic acids. In yeast and bacteria they comprise 50-120, respectively 80-160 g/kg of dry matter (McDonald *et al.*, 2011). The nucleic acids DNA and RNA comprises two groups of nucleotides, based on the nitrogenous bases in their structure, they are either purine or pyrimidine nucleotides (Griffiths *et al.*, 2012). High contents of purines seem to be the main issue with ingestion and subsequent metabolism of SCP according to a study made by Clifford and Story (1976), also reviewed by Carver and Allan Walker (1995). The metabolic pathways and end products of purines in different groups of animals is presented in figure 6.

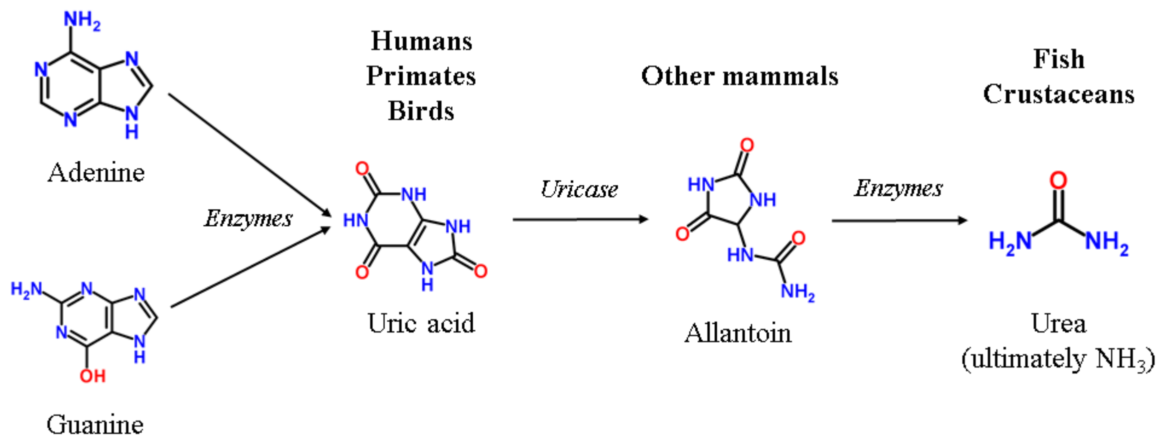

Figure 6 – Metabolism of purines, own interpretation based on information from Hayashi *et al.* (2000), McDonald *et al.* (2011) and Kratzer *et al.* (2014). Chemical structures with ChemSpider ID 185, 199, 744, 1143 and 1151 was downloaded from http://www.chemspider.com (accessed Apr 21, 2015).

Excessive amounts of uric acid in the blood leads to formation of urate crystals. These cause inflammation in the joints and may also deposit in soft tissue. Formation of uric acid stones in the kidneys has also been observed (Harvey & Ferrier, 2011). As seen in figure 6, the enzyme uricase is needed to convert uric acid further. Evolutionary studies shows that the genes coding for uricase have mutated over time, resulting in a less active or non-functional uricase in many mammals (Kratzer *et al.*, 2014). This is not the case for most fish and crustaceans (figure 6); furthermore they also convert the allantoin to urea, and in some species ultimately to NH_3, which both can be easily excreted. This knowledge presents unique possibilities for SCP as a feed in aquaculture, whereas it may be less suitable as feed for mammals. More detailed investigations on feeding fish with SCP based on yeast are summarized in section 3.3.

3.2.3 Nutrient requirements

From the groups of species mentioned in section 3.1, most important species will be investigated closer for their nutritional needs, summarized in table 1.

Table 1 – Nutrient requirement recommendations, adapted from NRC (2011). The table presents digestible energy (DE) as kcal/kg diet and digestible protein (DP) as % of total diet. The amino acids are presented as % of total diet if there is nearly 100 % bioavailability. Where there is no data reported by NRC, the cells are left blank.

Species	DE	DP	Arg	His	Ile	Leu	Lys	Met	Met Cys	Phe	Phe Tyr	Thr	Trp	Val
Atlantic salmon	4400	36	1.8	0.8	1.1	1.5	2.4	0.7	1.1	0.9	1.8	1.1	0.3	1.2
Common carp	3200	32	1.7	0.5	1.0	1.4	2.2	0.7	1.0	1.3	2	1.5	0.3	1.4
Rohu	3200	32	1.7	0.9	1.0	1.5	2.3	0.7	1.0	0.9	1.6	1.7	0.4	1.5
Tilapia	3400	29	1.2	1.0	1.0	1.9	1.6	0.7	1.0	1.1	1.6	1.1	0.3	1.5
Channel catfish	3000	29	1.2	0.6	0.8	1.3	1.6	0.6	0.9	0.7	1.6	0.7	0.2	0.8
Hybrid striped bass	4000	36	1.0				1.6	0.7	1.1	0.9		0.9	0.3	
Rainbow trout	4200	38	1.5	0.8	1.1	1.5	2.4	0.7	1.1	0.9	1.8	1.1	0.3	1.2
Pacific salmon	4200	40	2.2	0.7	1.0	1.6	2.2	0.7	1.1	0.9	1.8	1.1	0.3	1.2
Asian sea bass	4200	38	1.8				2.1	0.8	1.2					
Cobia	4200	38					2.3	0.8	1.1					
European sea bass	4000	40	1.8				2.2		1.1			1.2	0.3	
Japanese flounder	4000	40	2.0				2.6	0.9						
Grouper	4000	42					2.8							
Red drum	4000	36	1.8				1.7	0.8	1.2			0.8		
Yellowtail	4200	38	1.6				1.9	0.8	1.2					
Mean of fish[1]	3880	36	1.6	0.8	1.0	1.5	2.1	0.7	1.1	1.0	1.7	1.1	0.3	1.3
Kuruma prawn	4400	38	1.6	0.6	1.3	1.9	1.9	0.7	1.0	1.5		1.3	0.4	1.4
Fleshy prawn	3200	32												
Pacific white shrimp	3000	30					1.6							
Tiger shrimp	3000	34	1.9	0.8	1.0	1.7	2.1	0.7	1.0	1.4		1.4	0.2	
Mean of crustaceans[1]	3400	34	1.8	0.7	1.2	1.8	1.9	0.7	1.0	1.5		1.4	0.3	1.4
Mean of all species[2]	3779	36	1.7	0.7	1.0	1.6	2.1	0.7	1.1	1.1	1.7	1.2	0.3	1.3

[1]Calculated from species presented above.
[2]Calculated from all species presented in the table.

Further investigations of the data in table 1 indicate that DE is very similar between carnivorous fish species, from 4000 to 4400 kcal/kg diet, whereas Common carp, Tilapia, Rohu and Channel catfish show lower energy needs (from 3000 to 3400 kcal/kg diet). The same pattern is seen for DP were carnivorous fishes require from 36 to 42 % DP of total diet whereas for the other four species, 29 to 32 % of DP is sufficient. When it comes to amino acids, there is no clear pattern among species, either for fish or crustaceans. On the other hand, all crustaceans except the Kuruma prawn have a DE requirement between 3000 to 3200 kcal/kg diet. The need of DP is, as for DE, slightly higher for the Kuruma prawn than the other crustaceans.

To sum up, there are some major differences between the species in table 1 when comparing DE and DP, but for the amino acids, there are no clear patterns, apart from observing that lysine, leucine and arginine are the amino acids required in the highest amounts by all species.

Given the considerable diversity in fish digestive system and also nutrient requirements among the species used in aquaculture, it is clear that feeds need to be tailored optimally for individual groups and also the conditions for aquaculture. Factors such as digestibility and overall impact on health of the animal are of particular concern when proposing alternative feed ingredients, such as microbial biomass as a protein source.

3.3 Single cell protein (SCP) as fish feed

This section will summarize 11 earlier studies on feeding SCP based on yeast to fish. All studies focus on substituting FM, except for studies 1 and 6. Since all studies examined different parameters, only SCP inclusion level and growth will be presented along with relevant comments in table 2, summarized in no particular order.

Table 2 – Earlier studies on SCP as supplement for FM (and other ingredients) in feed for fish, own interpretation[1] of data. The growth column presents growth as g/day in the control group and then the percentage difference (against the control groups) where SCP was included by different levels.

	Study information	SCP, based on yeast	Inclusion	Growth
1	(Rumsey *et al.*, 1991) Rainbow trout	Control	0 %	0.11 g/day
		Brewer's dried yeast (BDY) Genessee Brewing Company, Rochester, NY	25 %	+ 10.87 %
			50 %	- 31.46 %
			75 %	- 80.44 %
	Study conducted for 70 days with a mean initial fish weight of 2.6 g. Inclusion based on the proportion of SCP in total feed, replacing casein and other ingredients. They concluded that 25 % of BDY has positive effects on growth while higher levels decrease the growth rate and even make the fish expel the pellets. Moreover, they also concluded that the high level of nucleic acids in SCP did not seem to have adverse effects on Rainbow trout, based on liver studies.			
2	(Øverland *et al.*, 2013) Atlantic salmon	Control	0 %	0.73 g/day
		Candida utilis	40 %	- 5.73 %
		Kluyveromyces marxianus	40 %	- 17.03 %
		Saccharomyces cerevisiae	40 %	- 20.74 %
	Study conducted for 89 days with a mean initial fish weight of 28 g. Inclusion based on the proportion of crude FM protein replaced by SCP. They concluded that *C. utilis* and *K. marxianus* can be used as a protein source for the Atlantic salmon, but more research is needed.			
3	(Hauptman *et al.*, 2014) Rainbow trout	Control	0 %	2.00 g/day
		Grain distillers dried yeast (GDDY) Archer Daniels Midland, USA	25 %	- 1.05 %
			37.5 %	- 1.05 %
			50 %	- 8.42 %
			62.5 %	- 8.77 %
			75 %	- 13.86 %
			87.5 %	- 17.54 %
			100 %	- 22.11 %
	Study conducted for 63 days with a mean initial fish weight of 22.1 g. Inclusion based on the proportion of digestible FM protein replaced by SCP. They concluded that nutrients from the GDDY was well digested and absorbed in the fish, whereas the reduced performance from higher levels of yeast is unclear.			
4	(Al-Hafedh & Alam, 2013) Nile tilapia Part A	Control	0 %	0.45 g/day
		Saccharomyces cerevisiae Grown on date processing waste	25 %	- 9.02 %
			50 %	- 24.50 %
			75 %	- 43.18 %
		Candida utilis Grown on date processing waste	25 %	- 13.17 %
			50 %	- 29.61 %

			75 %	- 33.28 %
	The same fish Part B	Control	0 %	0.77 g/day
		Saccharomyces cerevisiae Grown on date processing waste	25 %	- 10.16 %
			50 %	- 30.78 %
			75 %	- 48.76 %
		Candida utilis Grown on date processing waste	25 %	- 5.47 %
			50 %	- 31.68 %
			75 %	- 25.40 %
	Study conducted in two parts, the first (A) for 28 days with a mean initial fish weight of 15.39 g and the second (B), with the same fish, for 42 days with a mean fish initial weight of 25.14 g. Inclusion based on the proportion of crude FM protein replaced by SCP. They concluded that *S. cerevisiae* and *C. utilis* can replace 25 % of FM without any notable effects, and up to 50 % of FM if methionine is added (data not present in their study). Though, further studies are needed to improve utilization of yeast by testing other additives.			
5	(Omar *et al.*, 2012) Mirror carp	Control	0 %	0.48 g/day
		Yeast protein concentrate (YPC) From biofuel production AB Vista, United Kingdom	7.5 %	+ 7.68 %
			15 %	+ 24.31 %
			20 %	+ 31.44 %
			50 %	+ 8.46 %
	Study conducted for 56 days with a mean initial fish weight of 12.3 g. Inclusion based on the proportion of crude FM protein replaced by SCP. They concluded that up to 50 % of FM can be substituted by the YPC without any obvious adverse effects on health, and also that YPC may be suitable for Tilapia and Catfish. Further research is warranted to find optimal inclusion levels of YPC in major aquaculture species. They also expect more co-products from the growing bio-fuel industry that can be used by Aquaculture in the future.			
6	(Hatlen *et al.*, 2012) Atlantic salmon	Control	0 %	2.69 g/day
		Yarrowia lipolytica Y4305 Gen. mod. For EPA production	10 %	+ 4.14 %
			20 %	+ 4.27 %
			30 %	- 2.54 %
	Study conducted for 95 days with a mean initial fish weight of 179.1 g. Inclusion based on the proportion of SCP in total feed, replacing all fish oil and partially FM and other ingredients. They concluded that an inclusion level of *Y. lipolytica* up to 20 % was comparable with the control diet, also increasing EPA/DHA ratio in the fillet. However, more research is needed to increase bioavailability of nutrients in *Y. lipolytica*.			
7	(Oliva-Teles & Gonçalves, 2001) European sea bass	Control	0 %	0.34 g/day
		Brewer's yeast (BY) Gist Brocades, The Netherlands	10 %	+ 7.67 %
			20 %	+ 8.01 %
			30 %	+ 17.42 %
			50 %	+ 0.70 %
			50 %[2]	- 4.88 %
	Study conducted for 84 days with a mean initial fish weight of 12 g. Inclusion based on the proportion of crude FM protein replaced by SCP. They concluded that BY can replace up to 50 % of FM without any negative effects on growth, also protein retention was improved. However, addition of methionine to the 50 % diet had no beneficial effects. Finally, feed conversion ratio improved in diets with 10-30 % of BY inclusion.			
8	(Mahnken *et al.*, 1980) Rainbow trout Part A	Control	0 %	0.69 g/day
		"Viton" *Candida* sp. Grown on alkane substrate DIC, Japan	25 %	+ 1.60 %
			40 %	- 1.11 %
			40 %[3]	- 0.49 %
	Coho salmon Part B	Control	0 %	0.62 g/day
		"Viton" *Candida* sp. Grown on alkane substrate DIC, Japan	25 %	- 4.21 %
			25 %[2]	- 1.51 %
			50 %	- 7.65 %
			50 %[2]	- 3.72 %
			75 %	- 16.56 %
			75 %[2]	- 15.34 %
			100 %	- 27.93 %
			100 %[2]	- 22.21 %

	Study conducted in two parts, the first (A) for 162 days with a mean initial fish weight of 10.0 g and the second (B) for 196 days with a mean initial fish weight of 137.8 g. Inclusion based on the proportion of FM replaced by SCP. They concluded that Viton could be included up to 40 % with equal growth in part A. In part B, growth rate decreased depending on inclusion level, whereas in contrast, feed conversion ratio increased. In both parts, addition of methionine improved growth but not comparable to the control diet.			
9	(Güroy *et al.*, 2012) Rainbow trout	Control	0 %	0.76 g/day
		Organic NuPro™ All-tech Inc., USA	10 %	- 0.63 %
			20 %	+ 8.71 %
			30 %	+ 3.98 %
	Study conducted for 84 days with a mean initial fish weight of 4.0 g. Inclusion based on the proportion of crude FM protein replaced by SCP. They concluded that organically certified yeast can be an effective alternative to plant protein sources for organic Rainbow trout. However, n-3/n-6 fatty acid ratio decreased along with inclusion of NuPro™. Fish fed the 20 % diet grew significantly better than the reference group.			
10	(Lunger *et al.*, 2006) Cobia	Control	0 %	1.40 g/day
		Organic NuPro™ All-tech Inc., USA	25 %	- 1.76 %
			50 %	- 15.63 %
			75 %	- 53.52 %
			100 %	- 83.20 %
	Study conducted for 42 days with a mean initial fish weight of 11.5 g. Inclusion based on the proportion of crude FM protein replaced by SCP. They concluded that 25 % of the crude protein can be provided by NuPro™ in the diet. Higher inclusion levels indicated significant biological effects on the liver, viscera and muscle. Greater than 50 % resulted in detrimental effects on production.			
11	(Pongpet *et al.*, 2015) Thai Panga (hybrid)	Control	0 %	1.95 g/day
		Brewer's yeast (BY) Khon Kaen, Thailand	30 %	+ 5.64 %
			45 %	+ 18.97 %
			60 %	+ 5.13 %
			75 %	+ 3.08 %
	Study conducted for 242 days with a mean initial fish weight of 36.4 g. Inclusion based on the proportion of FM replaced by SCP. They concluded that BY can replace up to 45 % of FM, without any adverse effects. Moreover, the 45 % diet seem to have positive effects on growth performance and immune response.			

[1]All data in this table originates from the studies mentioned. Where necessary, original data have been converted to standardize the units and enable comparison among the different studies.
[2]Addition of methionine.
[3]Addition of feather-meal.

These studies collectively report both positive and negative effects of including yeast SCP in the diet. In studies with predominantly negative effects, the "magic" limit for minimal or potentially acceptable impact on growth appeared to be roughly 25-45 % of FM substitution. In studies with mostly positive effects, the optimal interval of FM substitution was generally between 20-40 %. From a wider perspective, all studies indicated that yeast SCP at appropriate inclusion levels could be a replacement for FM (also FO and casein, studies 1 and 6).

The most important conclusion from this survey is the fact that very few studies have been performed specifically addressing SCP as replacement for FM. Thus, more efforts should be directed to research the possibilities with SCP as feed for fish, especially focusing on why growth often is negatively affected by higher inclusion levels. Studies 2, 6 and 7 indicate that this may be caused by lower digestibility in feed with high inclusion levels of SCP. Studies 2, 4, 7 and 8 also identified that a low content of methionine in SCP might be another factor affecting growth. However, study 7 included methionine without any positive effects on growth. Moreover, effects on growth seemed to be influenced by yeast species and how the yeast biomass was treated before usage as feed ingredient in the studies. To sum up, improving digestibility and finding the right yeast species and also how to treat the biomass seem to be the most important research areas, concluded by this survey.

4 Laboratory trial

4.1 Materials and methods

4.1.1 Collection and preparation of the dairy waste substrate

Twenty 0.5 L aliquots of dairy wastewater were collected at Norrmejerier, a dairy industry in Umeå, Sweden. Collection was made from the hydrolysate tank in their biogas facility, the substrate circulates in this tank before further usage in the biogas facility. This particular batch of substrate was described as comprising mainly waste milk, which mostly is material from initial cleaning phases and products that do not meet quality standards (C. Hagelberg, personal communication).

The substrate was frozen directly after collection in a household freezer at -18°C, then transported to our lab using a Styrofoam container with freezer packs to prevent thawing. Arriving at SLU, the substrate was stored in a freezer at -20°C until the main trial was conducted.

Prior to the main trial, ten 0.5 L aliquots of substrate were thawed at 2°C for approximately 48 hours. The thawed aliquots were then pooled in a 5 L Erlenmeyer flask and thoroughly mixed using a magnetic stirring device. Triplicate aliquots (1.5 L) of this mixed substrate were used for the fermenter trial whereas the last 500 mL were stored in a sterile glass flask at 2°C for analysis (described in section 0).

4.1.2 Choice and preparation of yeast inoculum

Preliminary trials at Dept of Microbiology examining the growth of yeasts on dairy waste substrate in batch culture indicated that some species produced greater amounts of biomass (dry weight) on dairy wastewater (data not shown). *Kluyveromyces lactis* was identified as one of the top candidates in those trials, probably because it can digest both lactose and sucrose (Rodicio & Heinisch, 2013), which the dairy wastewater contains in relatively high amounts. The strain J469 of *K. lactis* from the culture collection at the Dept of Microbiology, SLU, was used for this trial, and was provided as a sub-culture, suspended in YPD-broth (20 g/L of bacteriological peptone and 10 g/L of yeast extract from BD (Le Pont-de-Claix, France) together with 20 g/L of glucose from Merck (Darmstadt, Germany)).

Pre-cultures were prepared by inoculating *K. lactis* into 2 × 100 mL YPD broth in baffled 500 mL Erlenmeyer flasks. The flasks where incubated on a rotary shaker (150 rpm) at 30°C for 24 hours.

After incubation, the flasks were pooled and 1 mL of pre-culture was transferred to an Eppendorf tube to determine the Absorbance (Abs). Abs_{600} was measured using different dilutions in an Ultrospec 1100 pro UV/VIS spectrophotometer (Biochrom Ltd., Cambridge, UK), and the inoculation volume to achieve a final Abs of 1.0 in the fermenters was calculated.

$$Abs_{Calculated} = Abs_{600}\ x\ dilution\ factor$$

$$Inoculation\ volume = \frac{Abs_{Final} * Substrate\ volume}{Abs_{Calculated}}$$

The inoculum volume of pre-culture as calculated above was pipetted into three sterile 50 mL plastic tubes. The inoculum was centrifuged using a JS-13.1 swinging bucket rotor with the Beckman Coulter Avanti J-26 XPI centrifuge (Brea, CA, USA) at 3000 *g* for 5 min, washed three times with sterile 0.9 M NaCl solution, and finally re-suspended in 10 mL of 0.9 M NaCl solution.

4.1.3 Fermentor setup

Three Jenny CP20 bioreactors with a max volume of 2.0 L from Belach Bioteknik (Stockholm, Sweden) were set-up and calibrated according to the manual. The system was controlled during fermentation by the software BioPhantom® 1.05 from the same company.

Each fermenter was filled with 1.5 L of substrate. The temperature was adjusted to 30°C, and the pH interval was set to 4.95-5.05, continuously regulated by automated addition of 3 M H_3PO_4 or 5 M NaOH. Aeration was set to 20 % pO_2, and the stirring speed was automatically regulated depending on the aeration, with an interval of 200-1000 rpm. The initial gas flow of O_2 was set to 1.0 L gas/L substrate per minute (vvm) and continuously manually adjusted to meet aeration, with a maximum of 2.0 vvm. Polypropylene glycol (a few drops < 0.1 mL in total) was added manually by a syringe to each fermenter to prevent foaming of the substrate.

When the fermenters parameters had stabilized (1-3 hours), *K. lactis* inocula from the 50 mL plastic tubes were added using a sterile syringe through the membranes on the top of each fermentor. The fermenters where then monitored throughout the whole run to see that BioPhantom® kept them stable based on set-up parameters.

4.1.4 Sampling

A 0.5 L sample was collected directly from the ten initial pooled aliquots of substrate, and stored in a sterile 500 mL glass flask at 2°C (see section 4.1.1).

During the fermenter run, 45 mL samples were collected from each fermenter in sterile 50 mL plastic tubes. Sampling was conducted every 3 hours from 0 hours (inoculation) until 24 hours, then every 6 hours, ending with the 48 hour samples.

After 48 hours, the fermenters were stopped, and the remaining substrate (approximately 1.5 L in total) was collected into sterile 500 mL glass flasks and stored at 2°C until further analysis, described in section 4.1.6.2.

4.1.5 Monitoring biomass increases during fermentation

4.1.5.1 Dry matter analysis of pelleted biomass

Samples (45 mL) from each fermenter were treated directly after collection. A 40 g aliquot from the 50mL plastic tube was transferred to a non-sterile 50 mL Beckman centrifuge tube, which was centrifuged using a JS-13.1 swinging bucket rotor with the Beckman Coulter Avanti J-26 XPI centrifuge (Brea, CA, USA) at 10000 *g* for 5 min.

After centrifugation, the supernatant was discarded, and the pellet re-suspended in 20 mL deionized water using a Vortex Genie® 2 (Scientific Industries, Inc., Bohemia, NY, USA). The purpose of this washing step was to remove lipids and soluble material which could interfere with the final analysis. The tube was filled with an additional 20 mL deionized water and centrifuged as described above. The tube was decanted after centrifugation and the pellet again re-suspended in 20 mL deionized water, after which the suspension was transferred to the original 50 mL plastic tube. Deionized water was added to reach the initial weight of 40 g, and the tube was shaken thoroughly by hand.

From each of the 50 mL plastic tubes, approximately 15 g was poured on a Sartorius aluminum sample pan (oil and fat free) and analyzed using the Sartorius MA45 moisture analyzer (Goettingen, Germany). The drying parameters were according to factory settings (105°C) and semi-automatic cut-off (less than 2 mg of weight loss under 24 s). The moisture analyzer readings were presented as g DM/kg substrate.

4.1.5.2 Estimation of yeast viable cell count

A 1 mL aliquot of the final suspension (after washing) as described in section 4.1.5.1 was collected in sterile 1.5 mL Eppendorf tubes and stored at 2°C for later estimation of the viable cell-count in each fermenter.

Fungal specific Yeast Malt Chloramphenicol (YMC) agar was prepared by dissolving 3 g/L of yeast extract, 3 g/L of malt extract, 5 g/L of bacteriological peptone and 16 g/L of agar from BD (Le Pont-de-Claix, France) together with 10 g/L of glucose from Merck (Darmstadt, Germany) and 0.1 g/L of chloramphenicol from Sigma-Aldrich (Steinheim, Germany) in deionized water with the aid of a magnetic stirring device. The medium was autoclaved for 20 min. After cooling, the agar was poured into 90 mm sterile petri dishes and left to dry overnight in a fume hood.

The final suspension was serially diluted in 0.9 mL sterile 0.9M NaCl solution, according to figure 7, resulting in dilutions from 10^{-1} to 10^{-10}.

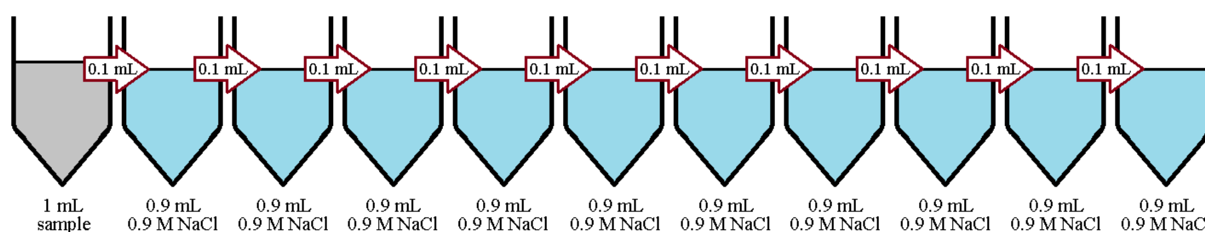

Figure 7 – Dilution series.

Each YMC plate was divided into 3 sectors and marked with 3 relevant dilution factors, and from each initial 1 mL sample, 6 dilutions was chosen to be plated. Plating was conducted by pipetting 3 × 10 µL spots from each dilution directly onto the agar. Plates were incubated at 20°C for 3 days, after which yeast colonies in each spot were enumerated.

4.1.6 Comparison of substrate parameters before and after the fermentation

4.1.6.1 Samples representing substrate before fermentation

The initial 0.5 L sample described in section 4.1.4 was sent to Agrilab AB (Uppsala, Sweden) for "gödselpaket" analysis. Parameters measured included dry matter, nitrogen (total, organic and ammonia), total carbon, C/N ratio, minerals (P, K, Mg, Ca, Na and S) and volatile solids.

To characterize the solid component of the unfermented substrate, i.e. that which could be pelleted by centrifugation, nine additional 0.5 L aliquots of original substrate received from Norrmejerier were thawed and mixed as described in section 4.1.1. This pooled substrate was centrifuged and the pellet washed and collected as described below in section 4.1.6.2. The dried pellet was sent to the Dept of Animal Nutrition and Management, SLU for proximate and amino acid analyses.

4.1.6.2 Samples at end of fermentation

The material from fermenters 2 and 3 was pooled in a 2 L non-sterile glass flask and mixed using a magnetic stirrer. It was necessary to pool this material to ensure that enough biomass (minimum 40-50 g, based on dry matter) was collected to do the analysis later on.

The contents of fermentor 1 could not be included in the analysis due to technical problems, see section 4.2 for details. From the pooled material approximately 6 × 240 g was transferred into six non-sterile 250 mL Beckman centrifuge bottles and centrifuged using a JA-14 fixed angle rotor with the Beckman Coulter Avanti J-26 XPI centrifuge (Brea, CA, USA) at 10000 *g*

for 5 min. After centrifugation the bottles were decanted and the supernatants were pooled in a non-sterile 2 L glass flask.

In a scaled-up procedure similar to that for dry matter analysis described in section 4.1.5.1, the pellet was re-suspended in approximately 100 mL of deionized water using a Vortex Genie® 2 (Scientific Industries, Inc., Bohemia, NY, USA). The bottles where filled with an additional 100 mL of deionized water and centrifuged as above. After centrifugation the pellets were transferred to sterile 50 mL plastic tubes and dried in a drying-oven at 105°C for 6 hours.

The pooled supernatant was sent to Agrilab AB (Uppsala, Sweden) for "gödselpaket" analysis, and the dried pellet material was analyzed at Dept of Animal Nutrition and Management, SLU, both as described in section 4.1.6.1.

4.2 Results and discussion

During the fermentation there were some major disturbances which interfered with the results, most crucial were connection problems with the temperature probes. Fermentor 1 killed the yeast before the last sampling at 48 hours, the temperature reached 46°C, which resulted in discarding the final results from fermentor 1. Fermentor 2 also had major problems in sustaining the correct temperature, resulting in too cold temperatures during the run, interfering with growth rate. Moreover, there were some minor occurrences, presented in Appendix A.

4.2.1 Biomass increase during fermentation

4.2.1.1 Dry matter analysis of pelleted biomass

Analysis of the results from the dry matter analysis as described in section 4.1.5.1 gives a growth curve, both as mean of all fermenters and as percentage increase in biomass. The curve is presented in figure 8 and seems to follow the typical growth characteristics of microorganisms. Raw data from the fermentation can be seen in Appendix B.

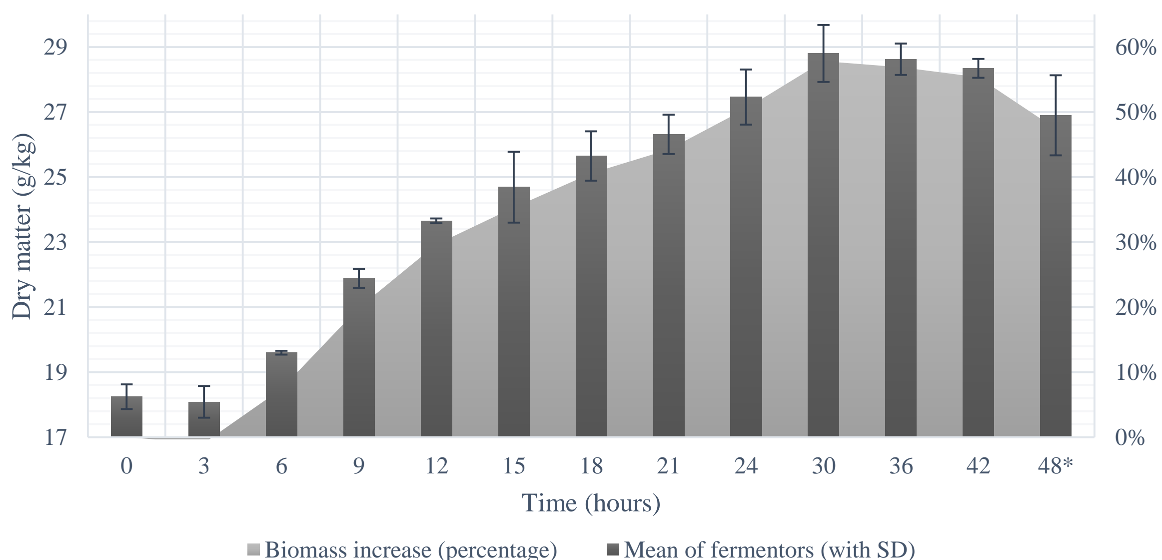

Figure 8 – Growth curve. *Fermentor 1 excluded at 48 hours due to technical problems.

From 0 to 3 hours there is no increase in dry matter, which indicates the lag phase, yeast is adapting to the substrate. From 6 to 30 hours there is a steady increase in dry matter, which indicates the growth phase, yeast is growing by consuming easy-to-digest nutrients. After 30 hours the growth stops and eventually starts to decrease, indicating the stationary phase, which may be induced by a shift in metabolism (yeast has consumed all easy-to-digest nutrients) or

from inhibition by production of primary metabolites. The further decrease in biomass after 30 hours may be a result of cell lysis and protein digestion due to altered metabolism (Waites, 2001). Unfortunately the viable cell count results in section 4.2.1.2 could not emphasize this due to unreliable results. These results indicates that it would be best to stop fermentation somewhere around 30 hours.

4.2.1.2 Estimation of yeast viable cell count

Estimations of viable cells/mL by enumeration of cfu/mL as described in section 4.1.5.2 is presented in figure 9.

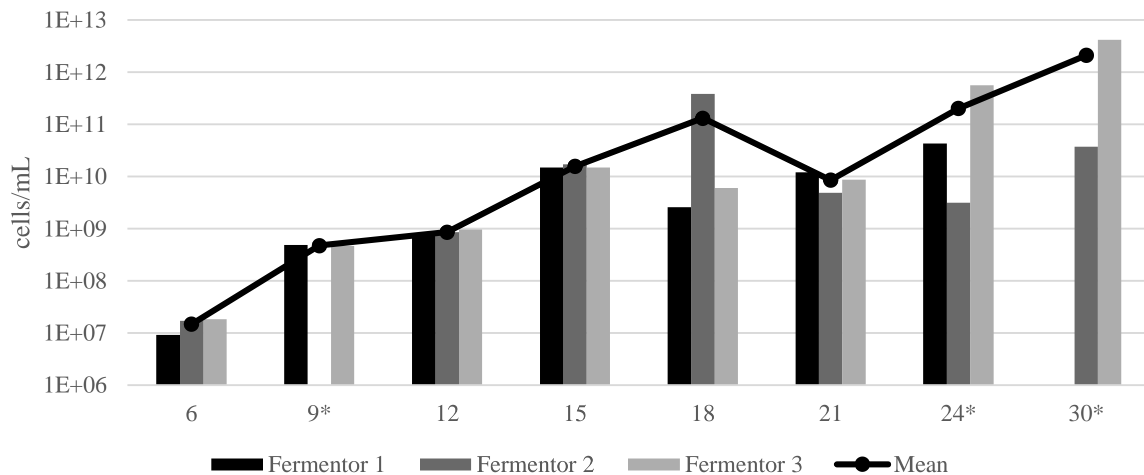

Figure 9 – Viable cells. *Plates missing or overgrown by mold.

Samples from 0 and 3 hours could not be plated as they were missing. At 9 hours one plate is missing and from 24 hours and forward the plates began to be overgrown by mold (further discussed below). However, samples from 6 to 15 hours show a somewhat clear pattern that corresponds to the dry matter analysis results shown in section 4.2.1.1. After 15 hours, the results showed a major non-pattern diversity between dilutions and fermenters, which makes them unreliable. This diversity may originate from wrong dilution and/or plating techniques, for example, from yeast cells clumping at the pipette tip or lack of emulsification in the substrate. Exchange of tips between plates and dilutions, and adding a surfactant to the diluent may be a solution to the problem.

Figure 10 – Mold colonies grown on MEA plates.

From the mold found on the plates there appeared to be 2 colony types present. Representative colonies were purified on MEA plates, presented after incubation in figure 10. Purified colonies were examined microscopically, and identified by Albina Bakeeva, Dept of Microbiology, based on sequencing the internal transcribed spacer (ITS) gene, as described in Båth *et al.* (2012). Microscopic and sequence-based analysis confirmed that all the isolates were *Geotrichum candidum*, a mold that is often found in milk and milk products. Recent studies also indicates that it has an important role in the ripening of many types of cheese (Samson *et al.*, 2010). This species was also previously isolated from an earlier batch of dairy waste substrate from Norrmejerier (J. Ohlsson, personal communication). Therefore, it seems likely that the source of the mold was the original dairy waste, rather than external contamination from the microbiology laboratory.

There is no clear reason why the mold was not present before the 24 hour plates. One probable reason may be that when the yeast started to enter the stationary phase, growth prerequisites became favorable for the mold.

4.2.2 Substrate parameters before and after fermentation

4.2.2.1 Biomass analysis

The most crucial result is whether the biomass is suitable as feed for fish or not, and if there has been any improvement in the nutrient content. Results presented in table 3 are based on the proximate analysis, performed on the washed and dried pellets from untreated substrate (Pre) and substrate after yeast treatment (Post).

Table 3 – Proximate analysis of the pellets by Dept of Animal Nutrition and Management, SLU. Abbreviations: Dry matter (DM), gross energy (GE), crude protein (CP) and total carbohydrates (TC).

	GE/kg DM		**Crude content (g/kg DM)**				**Minerals (g/kg DM)**					
	MJ	Kcal[1]	Ash	CP[2]	Fat[3]	TC[4]	Ca	Mg	P	K	Na	S
Pre	27.8	6640	25	618	299	58	0.8	0.1	5.2	1.3	0.5	4.6
Post	26.7	6377	29	473	306	192	0.6	0.7	7.7	6.4	0.4	3.5

[1]Calculated as 1 calorie = 4.1868 J, according to Alphonce & Pilström (2011).
[2]Based on Kjeldahl analysis with 6.25 as conversion factor.
[3]Analysed using the "EG-fett" method.
[4]Calculated as TC = 1000 - (ash + CP + fat), according to McDonald *et al.* (2011).

The proximate analysis indicates some major changes in the substrate after fermentation and the most crucial of them is the CP concentration which shows a decrease from 618 to 473 g/kg DM, more than 23 %. A decrease in CP would not be desirable, but if the increase in biomass is taken in consideration the result is positive. As seen in Appendix B, the biomass (dry matter) had increased by 47.4 % at the 48 hour sample. Calculations as seen below gives a total CP of 697 g in yeast treated substrate, concluding that there has been an addition of CP by 12.8 % to the initial amount, the yeast has converted ammonia to protein (see table 4).

$$Total\ CP = CP\ (g/kg\ DM) * Biomass\ increase = 473 * 1{,}474$$

$$Increased\ CP = \frac{Post\ CP}{Pre\ CP} = \frac{697}{618} \approx 1.128$$

Simultaneously, there was a major increase in TC from 58 to 192 g/kg DM, indicating that the yeast utilized soluble sugars from the substrate, primarily lactose, and produced β-glucans and other carbohydrates for their cell walls and other cellular components (Willey *et al.*, 2012). Finally there is also a major increase in Mg, K and P; whereas S has decreased.

To further draw any conclusions on the suitability as fish feed, the amino acid profile needs to be analyzed. The results from the amino acid analysis is presented in figure 11. The amino acid profile is presented in relation to the total CP content in both pre- and post-pellets.

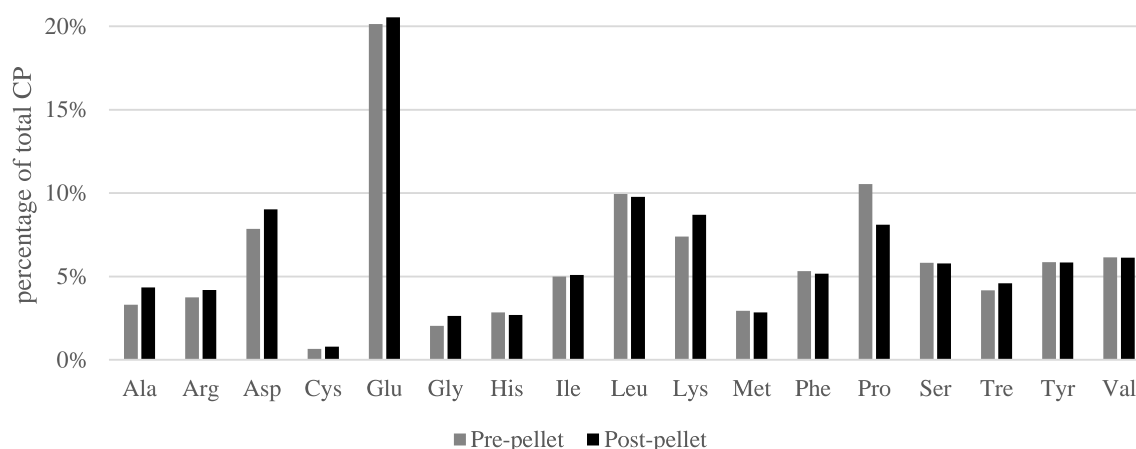

Figure 11 – Amino acid analysis from Dept of Animal Nutrition and Management, related to the total CP content of both pellets.

It is difficult to draw any accurate conclusions by just looking at the results in figure 11. Therefore, to further investigate the differences between the pre- and post-pellets, calculations on the differences between the samples is performed and presented in figure 12.

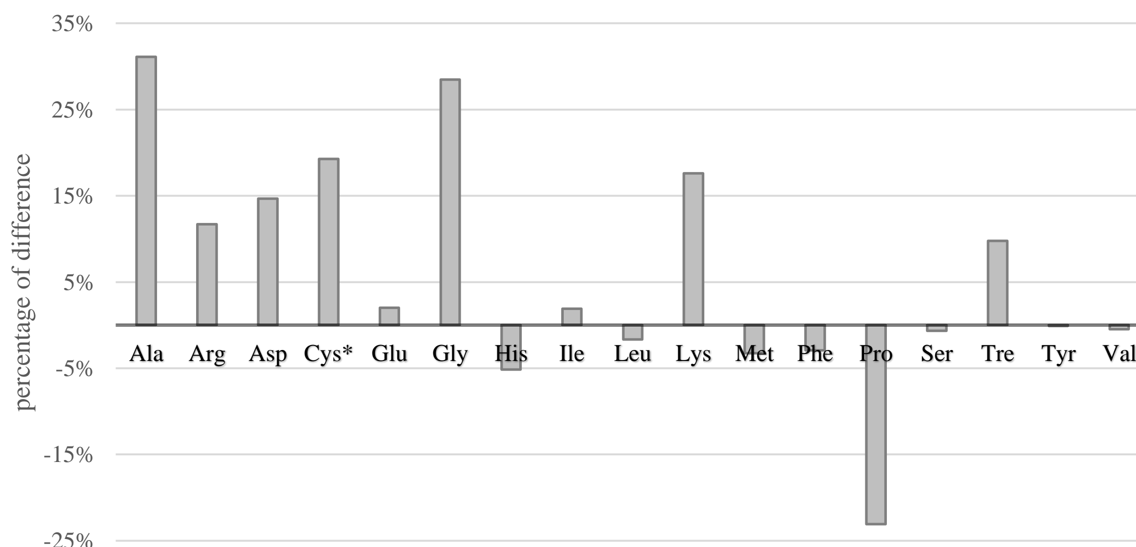

Figure 12 – Calculated percentage of difference for each amino acid (related to total CP) between pre- and post-pellets. *Cys comprises both cysteine and cystine.

There seem to be some major differences between the pellets in the analysis; alanine, cysteine, glycine and lysine has increased more than 15 % from the initial proportion. The only amino acid that shows a major decrease is proline, nearly 25 %. These results will be further discussed with regard to fish nutrition in section 5.2.

4.2.2.2 Biogas suitability

Apart from producing biomass there is also an interest in making the substrate more suitable for biogas production. Therefore, untreated substrate (Pre) and supernatant from the yeast treated substrate (Post) was analyzed. The results are shown in table 4.

Table 4 – "Gödselpaket" analysis from Agrilab AB (Uppsala, Sweden).

	Pre (g/kg)	**Post (g/kg)**	**Difference.[1] (g/kg)**	**Pre[1] (% of DM)**	**Post[1] (% of DM)**	**Difference[2] (%)**
Dry matter (DM)	99.0	26.0	-73.0			
Total nitrogen (N)	3.0	0.4	-2.6	3.03%	1.54%	-49.2%
Organic N	2.8	0.4	-2.4	2.83%	1.54%	-45.6%
Ammonium N	0.2	0.0	-0.2	0.20%	0.00%	-100.0%
Total carbon (C)	49.5	10.0	-39.5	50.00%	38.46%	-23.1%
Total Ca	0.59	0.74	0.15	0.60%	2.85%	377.6%
Total Mg	0.10	0.09	-0.01	0.10%	0.35%	242.7%
Total P	0.60	1.54	0.94	0.61%	5.92%	877.3%
Total K	1.85	1.82	-0.03	1.87%	7.00%	274.6%
Total Na	0.50	0.92	0.42	0.51%	3.54%	600.6%
Total S	0.20	0.07	-0.13	0.20%	0.27%	33.3%
C/N (ratio)	*16.5*	*22.5*	*6.0*			
Volatile solids (% of DM)	*93.4*	*63.4*	*-30.0*			

[1]Calculations based on original data.
[2]Calculation based on the calculated data.

From a first look on the results (first difference column), it seems like there has been a good reduction of carbon, nitrogen and sulfur. Moreover, there was an increase in the C/N ratio and also reduction of volatile solids, which seems to be good for biogas reactor stability (Schnürer & Jarvis, 2010). Further examination based on own calculations gives another story. Based on dry matter, the nitrogen was reduced by 49.2 % and carbon by 23.1 %. Mineral concentration in dry matter has increased, except for sulfur, the increase is of major proportions. However, further research is needed to draw more accurate conclusions.

The apparent discrepancy between the results in table 4 (pre-sample, 99 g DM/kg) with those presented in figure 8 and Appendix B (time 0 hours, 18 g DM/kg) can be explained by the washing step in preparation of the biomass pellet. The original substrate contains lactose and other soluble substances that will be removed during the washing step, resulting in non-comparable results.

5 General discussion

Maintaining food security will be a challenging task in the future, the need and desire for animal protein increase each day. Livestock production struggles with increasing climate impact and fisheries with decreasing wild fish supplies. Along with this negative development, aquaculture has made an impressive growth in the last 50 years. But as with all types of nutrient conversion, feed supply is a crucial part of production.

5.1 Aquaculture: Trends and insights

The trends in aquaculture show major dependencies on feeds that compete with human consumption; Tacon *et al.* (2011) identified 11 major groups of species that were dependent on external feed ingredients in various amounts. In 2008, these groups constituted around ½ of total aquaculture production and consumed more than 29 million tons of feeds that competes more or less with human consumption. Further investigation highlighted that only 1-4 % of SCP was included in diets, it must be possible to improve that number, and even produce the SCP on waste that we would otherwise not use.

One can wonder why there are not more research breakthroughs on exchanging feed ingredients to more sustainable alternatives. One possible answer may be that fish and crustaceans show substantial diversity in both digestive physiology (figure 5) and their nutrient requirements. However, further analysis on the nutrient requirements showed that there were not so many major differences among species. Summarizing these findings indicated that the most crucial research areas for alternative feeds are how the nutrients are absorbed and their availability in different feed sources.

During the literature study, it became clear that a number of studies had substituted FM (and FO) with SCP, but the problem was that all studies examined different parameters. To make them more comparable, some were not included in the survey, and with the final 11 studies, summaries and calculation of the data were performed to make the review in section 3.3. Working with all those articles supported the conclusion above, that availability of the nutrients in the feed seems to be the most important problem.

However, some of the studies showed very promising results, especially those made by Omar *et al.* (2012), Oliva-Teles & Gonçalves (2001), Güroy *et al.* (2012) and Pongpet *et al.* (2015). They substituted 20-40 % of the FM with positive effects on growth. One common denominator was that all used yeast derived from different by-products. Another promising study was that of Hatlen *et al.* (2012) which concluded that the genetically modified strain Y4305 of *Y. lipolytica*, a lipid accumulating yeast, could replace all FO along with some FM and other ingredients without negative effects on growth. This suggests that it should be possible to include greater amounts of SCP without negative effects.

5.2 Yeast treated dairy waste

Production of SCP from dairy waste seemed to be promising based on the primary results, as strain J469 of *K. lactis* increased the biomass content by almost 60 % (at 30 hours) as seen in figure 8. The laboratory analyses made by Agrilab, presented in table 4, also showed that there may be a beneficial effect on the biogas production if yeast is grown on the substrate before it is treated in the biogas reactor. However, further biogas trials on the post-yeast substrate is needed to determine the real impact of yeast treatment. It would also be interesting to know why the mineral content increased so substantially.

The proximate analysis (table 3) indicated a major decrease in CP concentration, which is not favorable. But in the same time it makes sense, because if no additional nitrogen is added to the

substrate, the yeast will not be able to produce more protein than there is nitrogen from the beginning. A quick comparison between the pellet analyses based on CP concentration indicates that it would be better to feed the pellet from untreated substrate to fish directly. But if the biomass increase is taken into consideration, there is actually a 12.8 % increase from the initial CP, indicating that the yeast has metabolized all ammonia. However, to take advantage of that extra protein, the fish (or crustacean) needs to eat more dry matter. Further evaluation of the post pellet results shows that the fat concentration and GE not has changed significantly. Moreover, when comparing the fat content in the post-pellet (306 g/kg DM) to that of different FMs (60-75 g/kg DM), there is a clear and major difference (McDonald *et al.*, 2011). However, if this is favorable in feeds designated for aquaculture, is unclear. There is also a major increase in TC which might comprise major amounts of β-glucans, as discussed earlier in section 4.2.2.1. These non-starch polysaccharides may have negative effects on nutrient uptake and digestive physiology (Sinha *et al.*, 2011). Finally, the mineral content shows an increase for Mg, P and K which seem to be more favorable for fish according to NRC (2011). However, the post-pellet shows a lower Ca/P ratio than the pre-pellet which is, in general terms, less favorable (McDonald *et al.*, 2011).

From the amino acid analysis in section 4.2.2.1, there can be seen some major differences in some of the amino acids between pre- and post-pellets. When comparing the results in figure 12 with table 1, two amino acids needed in the greatest amounts (lysine and arginine) have increased in terms of their relative proportions but a third amino acid needed in high amounts (leucine), has decreased. Moreover, some studies in section 3.3 indicated methionine as one of the growth limiting factors when feeding SCP to fish, the SCP produced in this study only shows a minor decrease in methionine. However, to draw any further conclusions, feeding trials on fish are needed.

6 Conclusions

Feed dependent aquaculture can be a future source for animal protein if new sustainable feed ingredients, not competing with human consumption, are developed. Fish and crustaceans shows wide diversities in their nutrient uptake, which points out that feeds need to be carefully produced to meet their individual needs. Based on the review in section 3.3, SCP shows great possibilities to replace FM in the future, and maybe also other feed ingredients, but more research is needed to improve digestibility of SCP, and the right yeast species to use.

Growing yeast on dairy waste shows promising results in this trial, there is a major increase in biomass, though, the CP concentration decreases. There can also be seen some positive effects on the amino acid profile. There also seem to be an improvement of the substrate for biogas production. To draw more accurate conclusions, more research is needed.

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Appendix

Appendix A

Distinct occurrences during the fermentation, starting with time of inoculation at 17:00.

Day 1	Comments
17:00	Foam starts to accumulate in the fermenters, this was prevented as described in section 4.1.3.
20:00	Noticeable acidic smell related to *K. lactis* fills the room.
22:00	Stirring speed based on aeration increases rapidly. Gas flow is manually increased in small steps from 1.0 to 1.5 vvm.
02:30	Noticeable smell of banana related to ester formation fills the room. Aeration gets harder to maintain, stirring speed continue to increase. Gas flow is set to 1.85 vvm on all fermenters. There are differences in keeping aeration steady between the fermenters.
04:30	Gas flow set to 2.0 vvm on all fermenters.
10:00	Increased the intervals for stirring speed on all fermenters from 200-800 rpm to 200-1200 rpm. Aeration is restored and steady, but pO_2 reached very low levels in Fermentor 1.
Day 2	**Comments**
14:00	2-3 mm thick lipid layer occurs in all tubes after centrifugation of 21 hour samples. Stirring speed is steady at 900-950 rpm in all fermenters.
17:00	Fermentor 2 is cold, there is a connection problem between the temperature probe and the system. After a quick fix the correct temperature is 14.1°C. The fermentor begins to heat up the substrate and stabilizes quickly at 30°C.
18:30	Fermentor 1 shows a major issue in finding the right stirring speed, there seem to be a glitch in the pO_2 probe. After decreasing the margins for stirring speed to be tighter, the problem is solved.
03:00	Fire alarm in the building, the fermenters was unharmed.
08:00	Fermentor 2 cold again, the connection problem is fixed.
14:00	Temperature in Fermentor 1 arose to 46°C, the system got no signal at all from the temperature probe which indicated 0°C, and resulted in maximum heating. The fermentor was stopped.

Appendix B

Data from the dry matter analysis described in section 4.1.5.1.

Time (h)	Fermentor 1	Fermentor 2	Fermentor 3	Mean of fermenters	Standard deviation	Biomass increase
0	18.268	18.614	17.855	18.246	0.380	0.00%
3	18.562	18.097	17.587	18.082	0.488	-0.90%
6	19.582	19.660	19.550	19.597	0.057	7.41%
9	21.982	22.103	21.553	21.879	0.289	19.92%
12	23.568	23.669	23.721	23.653	0.078	29.63%
15	23.434	25.348	25.289	24.690	1.088	35.32%
18	24.835	26.338	25.777	25.650	0.760	40.58%
21	25.748	26.240	26.954	26.314	0.606	44.22%
24	26.714	27.287	28.386	27.462	0.850	50.51%
30	28.300	28.287	29.810	28.799	0.876	57.84%
36	28.267	28.429	29.178	28.625	0.486	56.88%
42	28.680	28.151	28.209	28.347	0.290	55.36%
48[1]		27.772	26.029	26.901	1.232	47.44%

[1]Fermentor 1 excluded at 48 hours due to technical problems.

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