Bilag 1: Half-year report 4: 'Insect-based ingredients in aquafeed'

DTU Aqua
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Use of black soldier fly larvae meal (BSFLM) in fish feed

In the previous half year report, data was provided on the growth performance of Nile tilapia and rainbow trout fed diets with different size fractions of BSFLM (0-200µm, 200-400µm, and >400µm) including different concentrations of chitin (1.8, 2.7, and 15.4% DM, respectively). In the last six months, chitinase activity in the intestinal tract and nutrient digestibility was determined for both species. While there were no effects of BSFLM inclusion on fish performances during the relatively short digestibility trials, inclusion of the largest BSFLM fraction resulted in a reduced digestibility of nitrogen, dry matter, and nitrogen-free extract (i.e., carbohydrates) in both fish species compared to the other two size fractions and the experimental control diet. There were no similar effects of the fine and medium fractions in rainbow trout, while dietary inclusion of these two fractions slightly reduced the digestibility of nitrogen, dry matter and NFE in tilapia compared to the experimental control diet. The results indicate that chitin acts as an anti-nutritional factor, underlining the importance of reducing the chitin content in BSFLM to be able to include it in fish feed as an alternative protein source. Results from the two trials furthermore showed that both Nile tilapia and rainbow trout can digest chitin to some extent but that higher dietary chitin inclusion reduced chitin digestibility despite an increased chitinase activity in the intestinal tract. In summary, the results from the two trials indicated that mechanical sieving might be a sustainable method for removing chitin from BSFLM and that resulting BSFLM fractions with ≤ 2.7% chitin might be incorporated in fish feed as an alternative protein source replacing up to 25% of fishmeal or soybean meal. The maximum inclusion level, however, would need to be tested and confirmed in a dose-response growth trial.

Nutrient composition analysis

Several samples were sent by ENORM to DTU Aqua for nutrient composition analysis. This included new raw materials that potentially will be used as larval feed ingredients: pea protein concentrate, KMC Mos Mix, and delactose permeate (**Table 1**). Whilst pea protein concentrate and KMC Mos Mix have a composition that could be of interest as a feed ingredient, delactose permeate has a high ash content (8.5% as-is) which could hamper its possible inclusion in larval feed.

Table 1. Proximate composition of different raw materials sent by ENORM to DTU Aqua for proximate analysis on as-is basis. NFE+CF = nitrogen-free extract and crude fiber.

Parameter (% as-is)	Casein	Corn starch	Sugar beet molasses	Rye	Pea grits	Rapeseed cake	Sugar beet pellet	Nutrimix	Pea protein concentrate	KMC Mos Mix	Delactose permeate
Dry matter	94.8	88.5	69.6	88.6	88.5	89.6	89.3	98.8	23.9	11.3	26.2
Ash	6.8	0.1	11.9	1.9	3.1	6.1	3.4	89.8	4.1	1.0	8.5
Crude protein	80.7	0.0	9.2	8.8	19.1	28.0	8.5	0.0	7.2	1.4	1.9
Crude lipid	1.2	0.2	0.1	1.5	2.5	13.9	1.8	0.5	0.3	0.1	0.0
NFE+CF	6.1	88.2	48.4	76.5	63.7	41.7	75.7	8.6	12.3	8.7	15.8

Additionally, the nutrient composition of samples from BAADER and HOSOKAWA were analysed for potential chitin fractionation methods (**Table 2**). The lowest chitin content was found for product samples from BAADER, suggesting that this is the most efficient chitin fractionation method of the two. Fractionation into fine and coarse also showed to fractionate chitin, with lower chitin content in the fine fraction compared to the coarse fraction.

Table 2. Proximate composition of BAADER and HOSOKAWA trial samples (% as-is basis).

Trial	Sample	Dry matter (%)	Ash (%)	Crude lipid (%)	Crude protein (%)	Chitin (%)
BAADER	Product	23.9	2.0	11.6	6.4	0.2
	By-product	42.6	2.0	4.4	27.5	9.1
HOSOKAWA	1-4 fine	93.1	14.0	15.3	48.8	1.9
	1-4 coarse	93.7	9.0	10.5	53.1	22.6
	1-6 fine	94.8	13.4	14.9	50.1	6.6
	1-6 coarse	95.6	6.7	10.6	54.3	27.7
	2-1 fine	93.6	13.8	14.7	47.7	4.3
	2-1 coarse	94.2	9.1	11.9	50.9	22.4

Lastly, different samples (larvae, raw materials, and larval feed mixtures) were freeze-dried by DTU Aqua and sent back to ENORM for amino acid quantification at an external lab.

Feed optimisation for black soldier fly larvae trials

Under the supervision of DTU Aqua PhD student Manon Eggink, two trials were performed at ENORM by MSc student Irene Gil Donoso as part of her 5-month master student project on 'Feed optimization for black soldier fly larvae'. The first trial investigated the optimal dietary protein to energy ratio for black soldier fly larvae, whilst the second trial focused on the use of one of the alternative by-products (KMC Mos Mix, delactose permeate, pea protein concentrate) as larval feed.

The first study tested a protein to energy ratio of 4.8-20.8 mg protein kJ^{-1} gross energy, compared to a chicken feed control (11.1 mg protein kJ^{-1} gross energy). The study showed that a higher protein to energy ratio increased larval maximal body weight gain but also resulted in a lower survival. Considering the investigated parameters, the optimal protein to energy ratio was found to be between 11.2-14.4 mg protein kJ^{-1} gross energy.

Table 3. Ingredient inclusion and proximate composition of different diets tested for the pre-trial of trial 2.

Ingredient (% as-is)	**KMC20-**	**KMC20+**	**KMC30-**	**KMC40-**	**DLP5+**	**DLM5-**	**PP+**	**PP-**	**Mixture-**	**Mixture+**
Sugar beet molasses	-	-	-	-	-	-	-	-	-	-
Rye	6.6	3.4	5.8	2.8	-	3.3	5.7	10.6	3.0	-
Pea grits	16.8	17.1	16.3	18.1	22.0	21.6	14.5	12.4	17.5	17.5
Rapeseed cake	6.9	6.8	7.0	6.9	6.4	6.5	6.9	7.1	6.9	6.8
Sugar beet pellet	-	3.0	-	-	3.0	-	3.0	-	-	3.0
Delactose permeate	-	-	-	-	5.0	5.0	-	-	5.0	5.0
Pea protein concentrate	-	-	-	-	-	-	10.0	10.0	10.0	10.0
KMC Mos Mix	20.0	20.0	30.0	40.0	-	-	-	-	10.0	10.0
Water	48.9	48.9	40.2	31.5	62.9	62.8	59.1	59.1	46.8	46.8
Nutrimix	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8
Parameter (% as-is)										
Dry matter	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0
Ash	2.1	2.1	2.2	2.3	2.4	2.3	2.2	2.2	2.7	2.8
Crude protein	6.0	6.0	6.0	6.2	6.3	6.3	6.2	6.0	6.5	6.5
Crude lipid	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Nitrogen-free extract + crude fiber	20.4	20.4	20.3	20.0	19.8	19.8	20.0	20.3	19.3	19.2
Gross energy (MJ/kg)	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.4	5.4

Before the second study, a pre-trial was performed testing different by-products: pea protein concentrate, KMC Mos Mix, and delactose permeate (**Table 3**). It was found that the structure of the feed resembled chicken feed most with the inclusion of KMC Mos Mix together with sugar beet pellets. It was therefore decided that feeds for trial 2 should include different inclusion levels of KMC Mos Mix (20, 30, and 40%) and sugar beet pellets. Diets were formulated to resemble the macronutrient composition of chicken feed (**Table 4**).

Table 4. Ingredient inclusion and proximate composition of different diets tested for the pre-trial of trial 2.

Ingredient (% as-is)	**KMC20+**	**KMC30+**	**KMC40+**	**Chicken feed control**
Rye	3.3	2.6	0.1	
Pea grits	17.5	16.6	17.8	
Rapeseed cake	6.5	6.9	6.8	
Sugar beet pellet	3.0	3.0	3.0	
KMC Mos Mix	20.0	30.0	40.0	
Water	48.9	40.2	31.5	
Nutrimix	0.8	0.8	0.8	
Parameter (% as-is)				
Dry matter	30.0	30.0	30.0	30.0
Ash	2.1	2.2	2.3	1.9
Crude protein	6.0	6.0	6.2	6.1
Crude lipid	1.5	1.5	1.5	1.4
Nitrogen-free extract + crude fiber	20.4	20.3	20.0	20.5
Gross energy (MJ/kg)	5.5	5.5	5.5	5.5

Larvae fed the 30% KMC Mos Mix diet had a sligthly higher final body weight compared to the 20% or 40% inclusion diets (**Figure 1**).

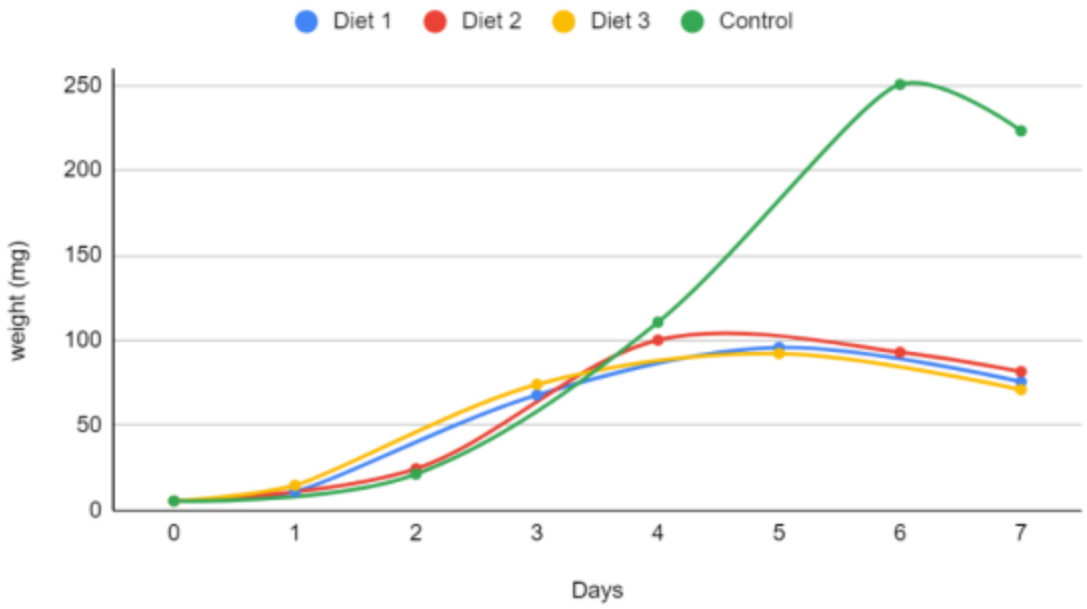

Figure 1. Mean larval wet body weight (mg larvae^{-1}) over the trial length for larvae fed diets including 20% (diet 1), 30% (diet 2), and 40% (diet 3) KMC Mos Mix compared to a chicken feed control diet. At the start of the trial, larvae were 5-days old. Figure is retrieved from Irene's master thesis.

In both trial 1 and trial 2, larvae fed the chicken feed control diet performed better than those fed the test diets. While feeds in the first trial were not optimized, feeds in the second trial were formulated to have a similar macronutrient and energy composition as chicken feed. Results therefore suggest that other factors than KMC Mos Mix play a role for larval performance such as micronutrient composition or physiochemical properties of the diets.

Teaching material for high schools

Manon participated in a teaching program for high school students aimed at designing teaching material to high school teachers. The teaching material informs students about sustainability and the use of insects as an alternative protein source for human and animal consumption. Furthermore, an experiment was designed by which students can determine chitin. Finally, a complementary film was made at ENORM and DTU supporting the teaching material.