

*Influence of fish meal reduction, algae addition, and ... (Sebastian Kaspers)*

## INFLUENCE OF FISH MEAL REDUCTION, ALGAE ADDITION, AND ENZYME USE IN SHRIMP FEEDS ON THE TISSUE COMPOSITION OF *Litopenaeus vannamei*

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### ABSTRACT

At the Leibniz-Center for Tropical Marine Ecology in Bremen, Germany, shrimps (*Litopenaeus vannamei*) fed feeds with reduced fish meal content, algae and enzyme additions were analysed for their tissue composition. Contents of protein, glycogen, total lipid, linoleic acid (LA), alpha linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) of the shrimp abdomen and of the respective feeds were determined. Reduction of fish meal (FM) content in shrimp feed and its substitution with "mash" (grain distillery waste-"Schlempe"), corn gluten, pea or coarse colza meal did not influence the tissue composition. Additional inclusion of an algae mixture out of *Spirulina* spp., *Phaeodactylum* spp., and *Tetraselmis* spp. to shrimp feeds as well as the single inclusions of *Phaeodactylum* spp. or *Spirulina* spp. had no effect on the tissue composition. Furthermore no improvement of the quality of the shrimps due to an enhanced digestibility of mash by enzymes (mixture of corolase, phytase, xylanase) could be detected. In contrast the quality of shrimps seemed to be negatively affected by enzymes in this study. These findings could contribute to making shrimp aqua farming more economic as reduction of feed costs without reduction of the quality of the shrimps would be a large benefit.

**KEYWORDS:** shrimp, tissue composition, fatty acid composition, feed addition, fish meal, microalgae

### INTRODUCTION

In order to improve feed management and reduce costs, several studies were done on the feed and nutrient requirements of fish and shrimp (Akiyama, 1992; Bureau *et al.*, 2000; Lupatsch *et al.*, 2008) and on the substitution of fish meal and fish oil (Amaya *et al.*, 2007a+b; Davis & Arnold, 2000; Davis *et al.*, 2004; Samocha *et al.*, 2004; Smith *et al.*, 2000; Suárez *et al.*, 2009). A summary of studies about fish meal replacement in shrimp feeds is also given in Fox *et al.* (2004).

One problem occurring with the substitution of fish proteins with plant proteins in aquaculture feeds is the low digestibility of plant proteins compared with those of marine origin. Although Akiyama *et al.* (1989) showed that apparent protein digestibility was not influenced by animal or plant feedstuff origin, Fenucci *et al.* (1982) observed differences in protein assimilation according to its source (animal or vegetable). To solve this problem, enzymes like amylase were effectively supplemented to shrimp diets to increase their di-

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gestibility and shrimp growth (Maugle *et al.*, 1983). Also Buchanan *et al.* (1997) stated an improved weight gain for *Penaeus monodon* through the addition of exogenous enzymes to a diet with canola meal. In contrast Divakaran & Velasco (1999) could not detect any positive or negative effects of enzyme addition to shrimp feed on the growth performance of the shrimp.

Another problem arising with the substitution of fish meal and fish oil in aquaculture feeds is the resulting lack of essential fatty acids (EFA) in the diets. EFAs are generally delivered from high-quality marine lipids which account for 50-100 g kg<sup>-1</sup> in commercial production feeds (Davis, 2005) and which are important in shrimp nutrition (Akiyama, 1992). The National Research Council (1993) depicted the fatty acid composition of common animal fats, fish- and vegetable oils. It is shown, that fish oil contains high amounts of the essential fatty acids EPA and DHA, which "are essential for normal growth and survival of marine penaeid shrimp" (Fox *et al.*, 2004), whereas animal fats (e.g. poultry fat) and vegetable oils (e.g. soybean oil), which are used as substitutes for fish meal and fish oil, are missing these EFAs. In order to compensate deficits in EFAs in aquaculture feeds and to cover the EFA requirements of the target organisms, marine algae rich in EFAs are added to the diets (Benemann, 1992; Duerr *et al.*, 1998; Gladue & Maxey, 1994; Ju *et al.*, 2009; Spolaore *et al.*, 2006).

Sriket *et al.* (2007) pointed out the important role which seafood plays in human diet and that black tiger shrimp and white shrimp contain high amounts of protein and polyunsaturated fatty acids (dominantly DHA and EPA) and therefore are a good source of these nutrients. It is further stated that "the compositions can vary with feed" and that "Differences in proximate composition might result in differences in nutritional value [...] of the shrimps."

EPA and DHA are important omega-3 fatty acids with positive effects on many health disorders (Liebke, 2007). LA and ALA are the parent compounds of the omega-6 and omega-3 fatty acids and the omega-6: omega-3 ratio is also important for human health (Simopoulos *et al.*, 1999). Today this ratio is 15-30:1 due to high levels of omega-6-fatty acids in our foods (for example in sunflower oil; Rüsing, 2007). For a healthy diet a ratio of 5:1 is recommended

as seen in the reference values for nutrient intake (German Nutrition Society (DEG) *et al.*, 2002).

The present study focuses on the concern, that alterations in feed compositions could affect the tissue composition of shrimps and therewith might change their nutritional value for human consumption. The Hypothesis to be tested is that the addition of mash, corn gluten, pea or coarse colza meal to shrimp feed, as well as the addition of micro algae or digestibility enhancing enzymes has an effect on the tissue composition of *Litopenaeus vannamei*. To investigate if the tissue composition changes due to the specific treatments, the protein, carbohydrate and total lipid content as well as the content of LA, ALA, EPA, and DHA in shrimp abdomen has been determined.

## MATERIALS AND METHODS

In this study, carried out at the Leibniz-Center for Tropical Marine Ecology in Bremen, Germany, samples of frozen shrimps (*Litopenaeus vannamei*) and feeds out of several feeding experiments (Table 1) were analysed for their nutrient composition. The feeding experiments covered the topics fishmeal substitutes, qualification of microalgae as feed addition and the qualification of mash as feed addition under the influence of enzymes. As fishmeal substitutes mash, corn gluten, pea and coarse colza meal were included in a commercial shrimp feed up to 150 g kg<sup>-1</sup> respectively. Thereby fishmeal was reduced up to 487, 200, 226, and 539 g kg<sup>-1</sup> respectively. In order to investigate the suitability of microalgae as feed addition, a mixture out of *Spirulina* spp., *Phaeodactylum* spp. and *Tetraselmis* spp. was included to shrimp feed up to 5, 10, and 30 g kg<sup>-1</sup>. Also either *Phaeodactylum* spp. or *Spirulina* spp. was included to the feed to 10 g kg<sup>-1</sup>. For the investigation of mash as feed addition under the influence of enzymes, enzymes (mixture of corolase, phytase, xylanase) and mash were included in the shrimp feed up to 17 g kg<sup>-1</sup> and 200 g kg<sup>-1</sup> respectively.

All post larvae shrimps were fed a commercial reference feed before changing to the test feeds, which were fed over 6-8 weeks. The experimental feed was the only source of food, cannibalism did not occur, neither did natural productivity because the water was treated

Table 1. Overview about feeding experiments, tested feeds and dry weight of the respective shrimp abdomen, FM= fish meal

Experiment	Feed	Replicate	Dry weight of abdomen [g]
Initial situation	Commercial reference – start (no FM substitutes)	1	1.0574
		2	1.8730
		3	2.2667
		4	0.5441
		5	0.5869
		6	0.3572
Fish meal substitutes	Commercial reference (no FM substitutes)	1	Not available
		2	0.9313
		3	1.8394
	Mash addition (150 g kg <sup>-1</sup> )	4	2.5559
		1	1.3837
		2	1.2405
	Corn gluten addition (150 g kg <sup>-1</sup> )	3	1.4471
		1	1.7657
		2	1.6269
	Pea addition (150 g kg <sup>-1</sup> )	3	1.4312
		1	1.0406
		2	1.2193
	Coarse colza meal addition (150 g kg <sup>-1</sup> )	3	1.3805
		1	1.3961
		2	1.4806
Microalgae addition	Reference (no algae)	3	1.3735
		1	1.1053
		2	0.7129
	Algae mix addition (5 g kg <sup>-1</sup> )	3	0.8021
		1	0.8257
		2	0.7609
	Algae mix addition (10 g kg <sup>-1</sup> )	3	1.0990
		1	0.6714
		2	1.0731
	Algae mix addition (30 g kg <sup>-1</sup> )	3	0.7775
		1	0.6812
		2	0.5120
	<i>Phaeodactylum</i> spp. addition (10 g kg <sup>-1</sup> )	3	0.8574
		1	1.0763
		2	1.0430
<i>Spirulina</i> spp. addition (10 g kg <sup>-1</sup> )	3	0.8159	
	1	0.7907	
	2	0.8689	
Mash and enzyme addition	No mash, Enzyme addition (17 g kg <sup>-1</sup> )	3	1.0470
		1	0.6120
		2	0.6779
	Mash addition (200 g kg <sup>-1</sup> ), Enzyme addition (17 g kg <sup>-1</sup> )	3	0.6481
		1	0.6501
		2	0.5170
		3	0.5706

several times. All tests were performed indoor in a closed recirculation system under the conditions stated in Table 2.

In order to eliminate experimental variables other than feed composition that could affect body composition e.g. developmental stage as well as stress due to starvation, temperature, salinity or light, these variables were maintained constant. Thus only shrimps with an equal start weight were used within each of the three experiments (Table 3), water parameters were monitored (Table 2) and automatic feeding devices were used for an even distribution of the feeding ratios over a day. At the end of the experiments shrimp were sacrificed by plunging them into iced water and stored at -40°C until being analyzed.

From the samples, the isolated and deveined abdomen of shrimps and the respective feeds were freeze-dried and homogenised before they were analysed for their protein-, carbohydrate- and total lipid content as well

as for their contents of linoleic acid (LA, 18:2 (n-6)), alpha linolenic acid (ALA, 18:3(n-3)), eicosapentaenoic acid (EPA, 20:5(n-3)) and docosahexaenoic acid (DHA, 22:6(n-3)).

The protein content of the samples was detected in a carbon/nitrogen (C/N) - analysis as described by Ehrenberger (1991). For this, the elemental analyser NA 2100 from Thermo and the standard SRM 1515 (apple leaf) was used.

As the main content of carbohydrates in shrimp tissue is glycogen (Murat & Serfaty, 1974), the glycogen content of the shrimp abdomen was examined. Thus, glucose was first released enzymatically from glycogen using amyloglucosidase from *Aspergillus niger* as described in Murat & Serfaty (1974). Afterwards the released glucose together with free glucose in the tissue was determined photometrically using glucose oxidase and peroxidase with ABTS as a chromogenic substrate (Bruss & Black, 1978).

Table 2. Experimental conditions and growth parameters for reference feeds in feeding experiments with *Litopenaeus vannamei* (Kunzmann & Pepler, unpubl. observ.)

Experiment	Microalgae addition	Mash and enzyme addition	Fishmeal substitutes
Age of shrimp	4-5 weeks	ca. 8 weeks	ca. 4.5 month
Developmental stage	Post larvae	Juvenile	Adult
Experimental period [days]	45	46	56
Feeding rate [g/d, pond]	2.4	2.4	1.5
Feeding rate [g/d, animal]	0.17	0.17	0.19
Animals/aquarium	14	14	8
Replicates	5	5	5
Water temperature [°C]	27.2-28	28-29.6	24.1-28.1
Salinity [g L <sup>-1</sup> ]	19.9-20.4	19.2-20.3	19.9-20.6
pH	7.96-8.13	7.1-8.12	8.28-8.35
NH <sub>4</sub> -N [mg/L]	0.1-0.2	0.2-0.7	0.06-0.08
NO <sub>2</sub> -N [mg/L]	0.03-0.05	0.06-0.08	0.02-0.08
NO <sub>3</sub> -N [mg/L]	144-178	123-155	218-230
Weight (start) [g]	ca.1.5 (1.49)	2.83-2.94 (2.9)	14.6-15.1 (14.8)
Weight (end) [g]	6.33-7.44 (7.3)	6.81-9.54 (7.7)	15.3-19 (16.65)
Biomass [g/m <sup>2</sup> ] (end)	359	294.1	437
Growth (weight (end) – weight (start)) [g]	4.9-6.2 (5.7)	4-6.8 (4.8)	0.8-4.4 (1.9)
Daily growth rate [g/d]	0.11	0.115	0.034
FCR	1.66	1.99	7.88
Mortality [%]	1.2	14	7.5

Table 3. Mortality and average weight of shrimps at start and end of the experiments (Kunzmann, unpubl. observ.)

Experiment	Feed	Start weight [g]	End weight [g]	Mortality [%]
Fish meal substitutes	Commercial reference	14.77	16.65	7.5
	Mash	14.97	16.22	7.5
	Corn gluten	14.54	16.05	2.5
	Pea	14.99	15.47	12.5
	Coarse colza meal	14.7	16.74	12.5
Microalgae addition	Reference (without algae)	1.49	7.29	1.4
	Algae mix (5 g kg <sup>-1</sup> )	1.53	7.51	1.4
	Algae mix (10 g kg <sup>-1</sup> )	1.51	7.27	7.1
	Algae mix (30 g kg <sup>-1</sup> )	1.5	7.46	14.3
	<i>Phaeodactylum</i> spp.	1.51	7.51	5.7
	<i>Spirulina</i> spp.	1.55	7.5	2.9
Mash + enzyme addition	No mash, enzymes	2.81	7.49	25.7
	Mash, enzymes	2.84	6.33	12.9

The total lipids of the samples were extracted as described in Hagen (2000) and then measured gravimetrically. The total lipid extracts of the samples were further analysed for their fatty acid composition by gas-liquid chromatography. Prior to the GC analysis the fatty acids were hydrolysed and derivatised to fatty acid methyl esters (FAMES) for better detection by the flame-ionisation detector (FID) (Kattner & Fricke, 1986; Peters *et al.*, 2006).

## RESULT AND DISCUSSION

The growth performances of the shrimp from several feed experiments are given in Table 4. There are significant differences in the tissue composition (Table 5) between the shrimps at the start of the experiment (commercial reference-start) and those at the end (commercial reference) due to growth of the shrimps during the experiment (Table 3). This confirms findings of Bureau *et al.* (2000) that

Table 4. Growth performances of shrimps *Litopenaeus vannamei* (Kunzmann & Peppler, unpubl. observ.)

Experiment	Feed variant	Growth [g/day/animal]	Significant differences between feed variants
Fish meal substitutes	Commercial reference	0.034	no
	Mash	< 0.03	
	Corn gluten	< 0.03	
	Pea	< 0.03	
	Coarse colza meal	0.036	
Microalgae addition	All feed variants	0.10-0.14	no
Mash + enzyme addition	All feed variants	0.08-0.11	no

Table 5. Average contents and standard deviation of the investigated parameters of the shrimps fed different fish meal substitutes and enzymes (n=3 for all feed variants except commercial reference (n=4) and commercial reference-start (n=6)). dw= dry weight, fa= fatty acids, enz= enzymes. Average values marked with a star are significantly different from the reference value (Mann-Whitney-Test, Pd7 0.05)

Diet	Glycogen [g kg <sup>-1</sup> dw]	Protein [g kg <sup>-1</sup> dw]	Lipid [g kg <sup>-1</sup> dw]	DHA [g kg <sup>-1</sup> fa]	EPA [g kg <sup>-1</sup> fa]	ALA [g kg <sup>-1</sup> fa]	LA [g kg <sup>-1</sup> fa]
Com. reference	10 ± 4	966 <sup>a</sup> ± 35	40 ± 1	82 ± 10	146 ± 13	14 ± 1	190 ± 6
Com. reference-start	30* ± 17	884* ± 27	45 ± 4	109* ± 11	134 ± 8	9* ± 1	139* ± 8
Mash	12 ± 6	934 ± 17	— <sup>b)</sup>	72 ± 8	143 ± 22	14 ± 2	206 ± 21
Corn gluten	8 ± 2	909 ± 22	42 ± 4	71 ± 3	140 ± 14	14 ± 1	196 ± 7
Pea	7 ± 6	864* ± 29	37 ± 3	75 ± 4	150 ± 5	13* ± 0	178 ± 9
Coarse colza meal	5 ± 4	876* ± 26	39 ± 6	69 ± 2	160 ± 23	12 ± 1	174 ± 15
No mash, enzymes	4* ± 1	859* ± 24	41 ± 4	94* ± 3	118* ± 5	13* ± 1	187 ± 7
Mash, enzymes	3* ± 1	945 ± 20	39 ± 5	92 ± 2	127 ± 7	12* ± 1	194 ± 5

a) Mean is overestimated due to some replicates with abnormally high protein contents

b) Value not available

Table 6. Average contents and standard deviation of the investigated parameters of the shrimps fed algae additions (n = 3). dw= dry weight, fa= fatty acids. Average values marked with a star are significantly different from the reference value (Mann-Whitney-Test, Pd7 0.05)

Diet	Glycogen [g kg <sup>-1</sup> dw]	Protein [g kg <sup>-1</sup> dw]	Lipid [g kg <sup>-1</sup> dw]	DHA [g kg <sup>-1</sup> fa]	EPA [g kg <sup>-1</sup> fa]	ALA [g kg <sup>-1</sup> fa]	LA [g kg <sup>-1</sup> fa]
Reference (no algae)	20 ± 3	915 ± 6	49 ± 6	99 ± 7	120 ± 6	16 ± 1	196 ± 3
Algae mix (5 g kg <sup>-1</sup> )	15* ± 1	909 ± 14	42 ± 4	104 ± 4	113 ± 5	15 ± 1	195 ± 5
Algae mix (10 g kg <sup>-1</sup> )	13* ± 1	919 ± 32	47 ± 5	101 ± 3	114 ± 3	16 ± 0	199 ± 3
Algae mix (30 g kg <sup>-1</sup> )	14* ± 4	948* ± 5	47 ± 1	99 ± 5	108* ± 8	15 ± 1	198 ± 4
<i>Phaeodactylum</i> spp.	14 ± 6	898 ± 25	46 ± 4	104 ± 4	115 ± 2	15 ± 1	192 ± 2
<i>Spirulina</i> spp.	16* ± 0	914 ± 14	50 ± 4	96 ± 2	110 ± 6	16 ± 1	201 ± 5

the chemical composition in penaeid shrimps varies with developmental stage. Thus the tissue composition and therewith the nutritional value of shrimps for human consumption differs depending on the developmental stage due to varying deposition of the several nutrients.

#### Fishmeal Substitutes

In the tissue composition of the shrimps fed the fishmeal substitutes (Table 5), no significant differences to the shrimps fed the commercial reference were found for the content of glycogen, lipid, DHA, EPA, and LA. Cheng *et al.* (2002) also stated no significant differences in crude fat content of Juvenile Pacific White Shrimp *Litopenaeus vannamei* when substituting fish meal by feather meal in shrimp diet.

When replacing fish oil with soy oil Cheng & Hardy (2004) also found no effect on the composition of LA, ALA, DHA, and EPA in the shrimp body of *Litopenaeus vannamei*. The difference of 1 g kg<sup>-1</sup> fatty acids in the ALA content between the shrimps fed the pea addition and those fed the commercial reference in the present study is not important in terms of human nutrition. Thus in contrast to expectations no disadvantages for the quality of shrimps could be detected when fed the fishmeal reduced diets. This finding could contribute to making shrimp aqua farming more economic, as reduction of feed costs without reduction of the quality of the shrimps would be a large benefit.

#### Algae Additions

Compared to the shrimps fed the reference food without algae additions, no significant differences in the contents of total lipid, DHA, ALA, and LA could be identified in the shrimps fed the algae additions (Table 6). The identified differences in the glycogen, protein and EPA contents between the reference group and the shrimps fed algae additions are very small and therefore negligible in terms of human nutrition. This indicates that the nutritional value of the shrimps for humans can not be improved by adding expensive micro algae to shrimp feeds, as expected.

Also Ju *et al.* (2009) stated that the fatty acid composition of shrimp tails (*Litopenaeus vannamei*) was not greatly affected by the addition of either whole algae or algae frac-

tions to a formulated diet and that no significant differences were observed in the content of DHA and EPA. However they observed an increased fatty acid content in shrimp tails when adding whole algae to the diet.

#### Enzyme Additions

Unlike expected the shrimps fed the enzyme additions did not show improvements in their tissue composition which would be relevant for human nutrition (Table 5). Generally the enzyme addition rather seemed to negatively affect the quality of the shrimps in this study. Divakaran & Velasco (1999) pointed out that added enzymes could alter the digestive enzyme activities in shrimps which could be an explanation for the negative effect of the added enzymes.

The data also suggest that there is no disadvantage for the quality of the shrimps when feeding mash at an inclusion level of 200 g kg<sup>-1</sup>, which would be a benefit for shrimp aqua culture concerning feed costs.

#### Diets

Contents of the investigated nutrients in feeds did not alter much, most were within the same range for the single nutrients (Table 7). Observed differences in the contents of the investigated nutrients could be due to the fact that they only represent one single measurement (n=1). Because of the low variation of the composition between the diets, no explicit reflection of the nutrient composition of the feeds could be detected in the shrimp tissue. When changing the amounts of fatty acids in shrimp feeds considerably, González-Félix *et al.* (2003a+b) detected a reflection of the fatty acid profile of the feeds in shrimp tissue. Also Lim *et al.* (1997) and González-Félix *et al.* (2002) detected a reflection of the fatty acid composition of the test diets in shrimp tissue.

#### CONCLUSIONS AND OUTLOOK

The present study focused on concerns, that alterations in compositions of shrimp feed could affect the tissue composition of shrimps and therewith might change their nutritional value for human consumption. The Hypothesis was that the addition of mash, corn gluten, pea or coarse colza meal to shrimp feed, as well as the addition of micro algae or digestibility enhancing enzymes has an effect on the tissue composition of *Litopenaeus vannamei*.

Table 7. Contents of investigated parameters of feeds (n= 1). dw= dry weight, fa= fatty acids, enz= enzymes. No replicates, because manufacturer given values were cross-checked

Diet	Glycogen [g kg <sup>-1</sup> dw]	Protein [g kg <sup>-1</sup> dw]	Lipid [g kg <sup>-1</sup> dw]	DHA [g kg <sup>-1</sup> fa]	EPA [g kg <sup>-1</sup> fa]	ALA [g kg <sup>-1</sup> fa]	LA [g kg <sup>-1</sup> fa]
Commercial reference	220	433	115	68	66	37	270
Mash	161	404	75	45	42	43	377
Corn gluten	194	415	86	58	51	39	328
Pea	158	392	120	57	50	42	318
Coarse colza meal	176	409	128	53	51	45	332
No mash, enzymes	222	284	95	58	48	44	352
Mash, enzymes	164	326	89	61	50	41	356
Reference (no algae)	232	374	87	62	53	40	314
Algae mix (5 g kg <sup>-1</sup> )	216	397	85	65	54	39	309
Algae mix (10 g kg <sup>-1</sup> )	189	374	103	67	60	39	307
Algae mix (30 g kg <sup>-1</sup> )	190	354	75	68	58	39	307
<i>Phaeodactylum</i> spp.	212	435	99	60	53	39	313
<i>Spirulina</i> spp.	180	395	86	64	53	38	306



The results of this study showed, that the investigated alterations of the feed composition did not have an influence on the tissue composition of the shrimps and thus did not change their quality in terms of their nutritional value for humans. The investigated algae and enzyme additions were not of immediate benefit for the quality of the shrimps in this study. However, there are many more algae species and enzymes, which might have direct positive effects. Also the effect of algae and enzyme additions on the quality of further shrimp species could be tested. Finally, even if there are no direct benefits for shrimp quality, long term effects on the immune system of the shrimps are highly likely when feeding algae additions, which should be object of a further study. The substitution of fish meal in shrimp feeds by cheaper alternatives like mash, pea, corn gluten or coarse colza meal is promising. It would be interesting to find out whether fish meal can be substituted to even higher amounts without affecting the quality of the shrimps.

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