TRIAL REPORT : SEABASS

Determine optimal levels of fishmeal/fish oil replacement with soy products (soybean meal, soybean oil and soy protein concentrate) in practical feeds for European seabass (*Dicentrarchus labrax*)

Scientific team

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1 Objective

The results from the trials performed during 2007 for seabass indicated that the species is more sensitive to fishmeal/fishoil replacement than Gilthead seabream. In the treatments where FM was providing 35% of the protein, daily growth rate and food conversion were negatively affected compared to a control diet containing 65% FM protein. It was concluded that 40% FM protein was the main focus for the formulations of the 2008 program.

Another point of study was to check the effect of the type of the SPC, more precisely the type of SPC depending on the processing applied to the soybean meal.

The diets evaluated in this trial were:

1. **FM65**, high quality reference feed, 65% of dietary protein provided by FM (prime S.Am).
2. **FM40A**, mixture of SBM and SPC1 (65% protein); 40% of the protein provided by fishmeal.
3. **FM40B**, mixture of SBM and SPC2 (60% protein); another type of SPC to check the effect in function of SPC characteristics
4. **FM40C**, mixture of fermented SBM and SPC1, to check the effect of the different soybean protein sources
5. **FM40D**, mixture of SBM and SPC1 plus an extra supplementation of digestibility and palatability enhancers.
6. **FM40E**, mixture of SBM and SPC1, using a higher quality fishmeal, LT fishmeal.

Detailed formulation is presented in Table 1.

Table 1: Feed formulations:

ASA 08 BASS FORMULAS	FM 65	FM40A	FM40B	FM40C	FM40D	FM40E
S Am Prime Fishmeal (67)	42,5%	26,2%	26,2%	26,2%	26,2%	
Scandinavian LT fishmeal (66)						**26,5%**
Fish Oil	15,7%	10,3%	10,2%	10,6%	10,2%	9,3%
SBM (Provasoy Cargill) (50)	18,3%	**20,0%**	**20,0%**		**20,0%**	**20,0%**
Fermented SBM (HP 310 Hamlet Protein) (56)				**17,6%**		
SPC (Danpro A Solea) (65)		**12,5%**		**12,5%**	**12,5%**	**12,5%**
SPC (Imcosoy 60 Imcopa) (60)			**13,6%**			
Soya Oil		6,0%	6,0%	6,0%	6,0%	6,0%
Corn Gluten (60)	4,0%	6,0%	6,0%	6,0%	6,0%	6,0%
Wheat Gluten (78)	3,1%	3,8%	4,0%	3,5%	3,7%	3,7%
Wheat Flour	15,9%	12,1%	11,0%	14,6%	11,2%	13,0%
Vit-Min Premix	0,50%	0,50%	0,50%	0,50%	0,50%	0,50%
Mono Ca Phosphate		0,40%	0,40%	0,40%	0,40%	0,40%
L Lysine (78%)		0,20%	0,20%	0,20%	0,20%	0,20%
Methionine (99%)		0,10%	0,10%	0,10%	0,10%	0,10%
Palatability/digestibility enhancers (INVE Aquaculture)		1,80%	1,80%	1,80%	**3,00%**	1,80%
SUM	100,0%	100,0%	100,0%	100,0%	100,0%	100,0%
Dry Matter (%)	91,56	92,08	91,94	91,94	92,18	92,60
Crude Protein (%)	45,00	45,00	45,00	45,00	45,00	45,00
Crude Fibre (%)	1,02	1,61	1,65	1,66	1,60	1,89
Crude Fat after Hydrolysis (%)	20,00	20,00	20,00	20,00	20,00	20,00
Crude Ash (%)	8,35	7,46	7,24	7,36	7,54	7,50
Lysine (%)	2,67	2,68	2,71	2,70	2,70	2,88
Methionine (%)	0,96	1,00	1,01	0,99	1,02	1,11
Protein from fishmeal (% of tot protein)	0,65	0,4	0,4	0,4	0,4	0,4
Protein from Soya (% of tot protein)	0,2	0,4	0,4	0,4	0,4	0,4
Protein from Wheat Gluten (% of tot prote	0,054	0,066	0,069	0,061	0,064	0,064
Protein from Corn (% of tot protein)	0,058	0,087	0,087	0,087	0,087	0,087
Phosphorus (%)	1,00	0,97	1,01	1,02	1,03	1,03
Vit E (ppm)	250	250	250	250	250	250
Vit C (ppm)	250	250	250	250	250	250
WEIGHT (%)	100	100	100	100	100	100

2 Materials and methods

2.1 Feed Preparation

Feed preparation was subcontracted to INVE Aquaculture N.V. Belgium which is producing the feeds in their pilot extrusion plant for specialty fish feed, including:

- Fresh ingredients from the factory stock passed through INVE quality control (physical, chemical, legal aspects..)
- Pulverization of coarse ingredients
- Mixing ingredients with double shaft paddle mixer
- Cooking-extrusion/drying on twin-screw Buhler extruder line on required pellet size
- Vacuum coating of oil phases
- Quality control (including analysis of feed specifications : C Protein/C Fat after hydrolysis/ash/moisture)
- Packaging and transport by courier

2.2 Experimental design

The set up used for the trial consisted in a R.A.S (recirculating aquaculture system) with 18 cylindrical fibre glass tanks with a working volume of 600 L, with well seawater of 22 ± 1°C and salinity of 36 ± 1 g/l. The system had a renewal of 10-15 % per day depending on the water quality values and a flow rate of 8-9 l/tank/min. Initial density was 4 Kg/m^3, with 27 individual per tank. The tanks were connected to a biofiltration unit made of 3 tanks with different biofilter substrates (rigid plastic mesh and moving plastic beds) and to a protein skimmer; a swirl separator is also part of the RAS as well as a sand filter. Photoperiod was set to have 12 h of light (12L/12D); feeding (with automatic belt feeders) lasted also 12 h (8.00 h to 20.00 h). Experimental fish, *D. labrax* juveniles were obtained from a local hatchery and grown up in our facilities. Trial duration was 14 weeks (3 weeks acclimation and 11 weeks of experimental diets). Water quality (T.A.N, nitrite) was checked three times per week. Temperature and dissolved oxygen was checked daily.

- Feeding

The fish were fed with automatic belt feeders, depositing the daily feed ration on the belt feeder at first hour in the morning (working days and Saturdays). Automatic belt feeder was set to work during 12h /day (8.00- 20.00) together with illumination. The first three weeks the fish have received an acclimation diet, the same for all the tanks.
The daily feed ration was calculated following feeding tables for this size of fish. Each tank had received a fixed % feeding ration in function of its biomass; the same % of tank biomass was applied to all the tanks.
Not eaten pellets were collected per tank twice per day and the feed intake was corrected accordingly.

- Stocking and sampling

For all the handling of the animals, phenoxyethanol has been used as anaesthetic. Before stocking fish were sampled (weight in water) to determine size distribution. Stocking has been done sequentially. Diets were assigned to the tanks at random.
Seabass had an acclimation period of three weeks. Once experimental feeds were provided, sampling happened every two weeks (except for the first period which was 19 days); for the sampling, all the fish/tank have been weighed in groups and carefully observed to check the health status of the animal. From these samplings, average weight has been determined and feed ration adjusted accordingly every two weeks.
At final sampling fish have been weighed individually and 3 individuals per tank (9 per treatment) have been picked randomly for dissection to determine HSI/VSI index and filet collection for further analyses; these 3 fishes per tank were used for histological examination of the intestine epithelium (9 fish per diet).

- Evaluation Parameters

- Specific Growth Rate (SGR): Ln(final weight/initial weight)*100/days of feeding), (%/day)
- Survival , %
- Food conversion ration FCR (feed intake/wet weight gain)
- Filet composition (Protein, Fat after Hydrolysis, Moisture)
- Hepatosomatic index HSI (liver weight*100/total weight) and Viscerosomatic index, VSI (viscera weight*100/total weight)
- Filet index, FI (right side filet weight*100/total weight)
- Gut histology

3 Results

Water quality parameters were maintained within the range of acceptable values for the culture of the species.

Growth results

Seabass have been in the experimental system during 14 weeks; the first three weeks have been considered as acclimation period. Experimental feeds have been provided during the next 11 weeks. The data presented are the average of three replicates per treatment.

After the 11 weeks of experimental feeding, no significant differences were observed among the treatments (ANOVA, *P>0.05*). Growth curves were very similar for all the treatments, although the FM65 diet had the highest SGR (0.81 %/day).

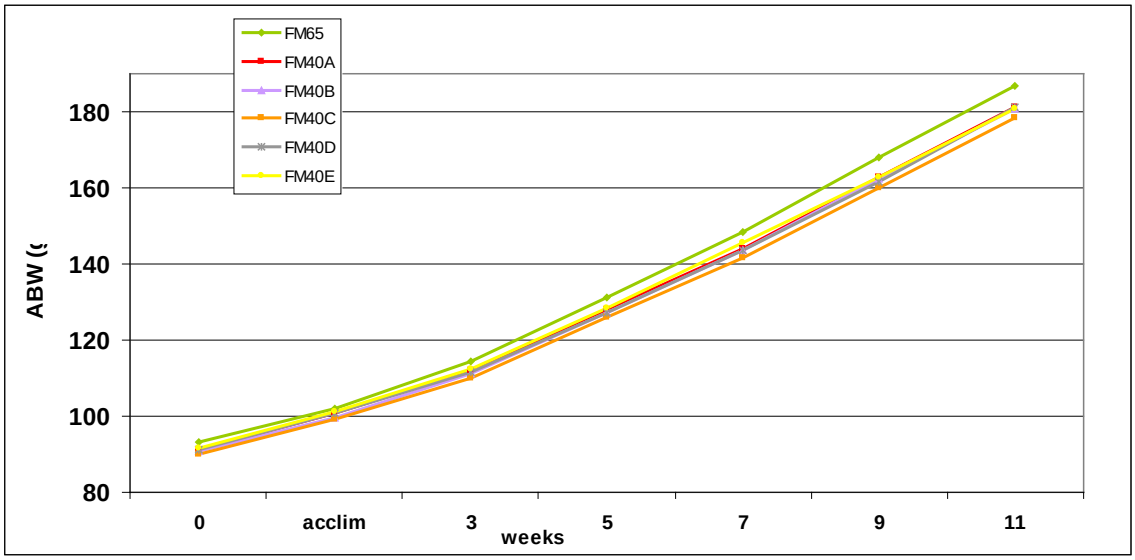

Fig. 1- Growth curve of seabass fed during 11 weeks the experimental diets (first three weeks were acclimation period; first period of feeding experimental diets lasted three weeks too)

Specific growth rate and food conversion ratio were not affected by the type of diet. No significant difference (*P>0.05)* was found for survival, total feed intake, feed intake as percentage of average body weight per day or weight gain among treatments (Table 2).

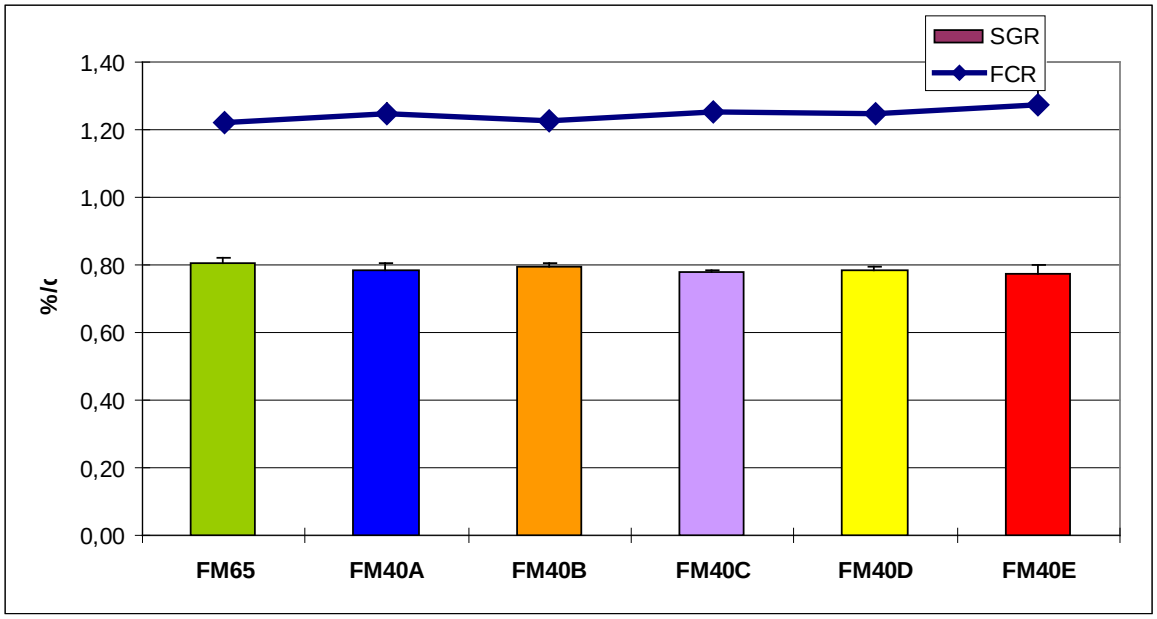

Fig. 2- SGR and FCR of seabass fed experimental diets during 11 weeks.

Concerning organosomatic indexes, HSI, VSI and FI (Table 2), all the diets showed a similar values and no significant difference (ANOVA, *P>0.05)* was found among the diets. However if we compared the HSI of the FM65 treatment with the HSI of diets FM40B, FM40D and diet FM40E, they are 12.02%, 13.10% and 7.5% lower respectively. Concerning, the VSI, FM40B and FM40D had 2.89% and 9.44% lower value than the FM65 diet.

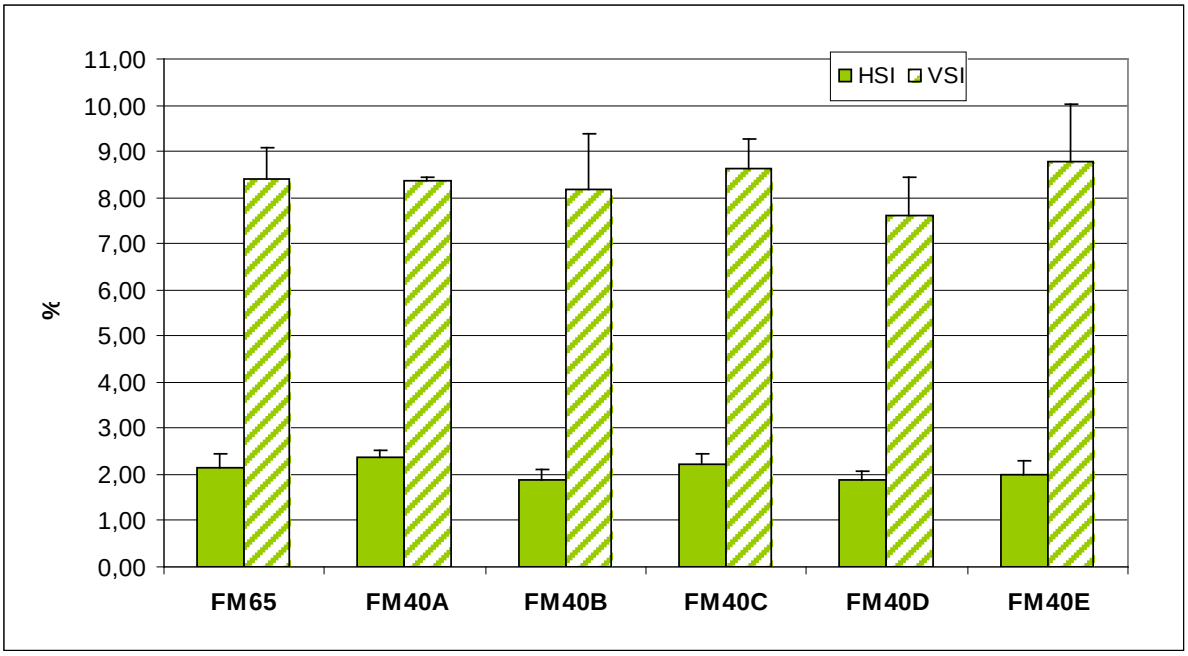

Fig. 3- HSI and VSI of seabass fed experimental diets during 11 weeks. Represented values are the mean and standard deviation of 9 replicates.

Filet analyses

Concerning to proximal composition of the seabass filet, the results are presented in Table 3. No significant difference was found among the different treatments in filet composition (ANOVA; *p<0.05)*. Contrary to what was found in the seabream trial, in the case of seabass the lowest value for Crude fat corresponds to the control diet, FM65, showing once more the differences between the two species when fed vegetables ingredients.

Table 3- Proximal composition of the seabass filet; data are expressed as % wet weight.

Diet	FM65	FM40A	FM40B	FM40C	FM40D	FM40E
Dry matter	30.73±1.52	30.77±1.44	30.37±0.42	30.87±0.55	29.67±1.91	29.93±0.32
Crude Protein	19.37±0.49	19.93±0.55	19.77±0.25	19.33±0.35	19.53±0.06	19.43±0.74
Crude fat	7.27±1.29	8.23±2.93	7.87±0.47	8.57±0.72	7.40±2.35	7.80±0.10
Crude ash	1.57±0.12	1.47±0.06	1.45±0.09	1.57±0.08	1.48±0.07	1.54±0.06

Histological analyses

In a general overview of all the samples of his study, it can be pointed out that no significant lesions have been found in these samples. However, some differences among samples have been noticed in terms of presence of supranuclear material in the enterocytes of the posterior intestine (indicating protein absorption) and also, in the level of presence of inflammatory cell infiltration in the mucosa and submucosa of the posterior and central part of the intestine. The presence of diffuse inflammatory cells in the these parts of the intestine are normal finding in this species, but the observation of different levels of presentation of these features in the samples made necessary a more precise evaluation. Results of this evaluation have been expressed in Fig. 4, but it has to be taken into account that the graph is based on quantification of qualitative observations, thus no statistical treatment is possible. The only intention is to show the differences observed among diets in a condensed way. Images of this histological study are included in a separate document.

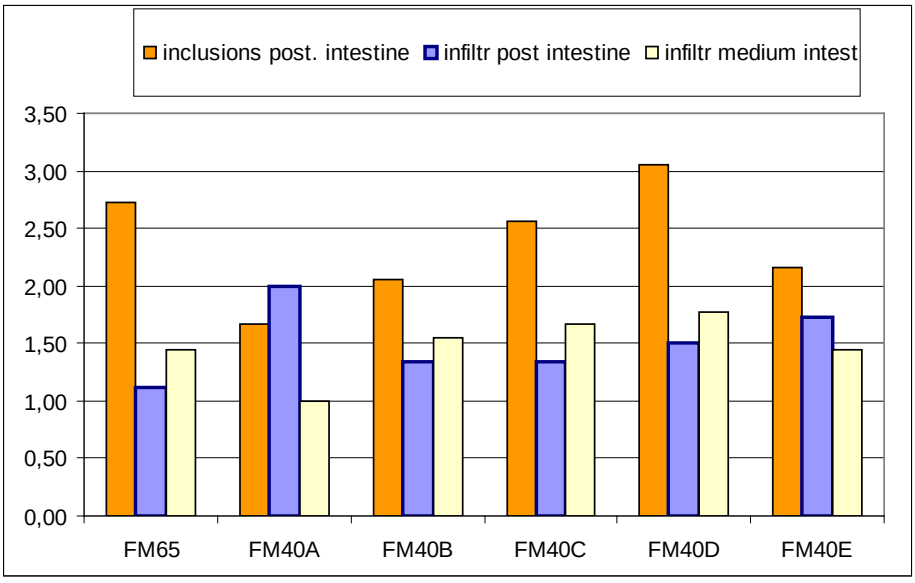

Fig. 4- Presence of the different observations on the intestinal epithelium samples from seabass fed the different diets. Observations have been scored in a 0 to 4 scale, indicating 0: not observed, 1: very light; 2: light; 3: moderate; 4: intense.

4 Conclusion

The results of the present trial show that European seabass of 100 g body weight, fed a diet with only 40 % of the dietary protein coming from FM and a mixture of SBM and SPC, do not show significant differences in growth and food conversion compared with seabass fed standard commercial diet, FM65, used as a control diet in this trial. Albeit not significantly different with the FM65 diet, the best results were obtained using FM40B diet, which includes a source of SPC with 60% protein (5% lower than the other source tested in this trial).

The relative difference for the replacement was lowest in the FM40B diet (Fig. 5), only 1.18% lower SGR than FM65. No major advantage was observed with the inclusion of fermented SBM, diet FM40C. It was unexpected the result of the FM40E diet, which included a higher quality FM and as it can seen in the graph, the relative performance with the control was 4.25% lower SGR and 4.35% higher FCR compared with FM65 and the lowest of all the tested diets. Likewise, no advantage was observed with diet FM40D, which had a higher inclusion of palatability and digestibility enhancers.

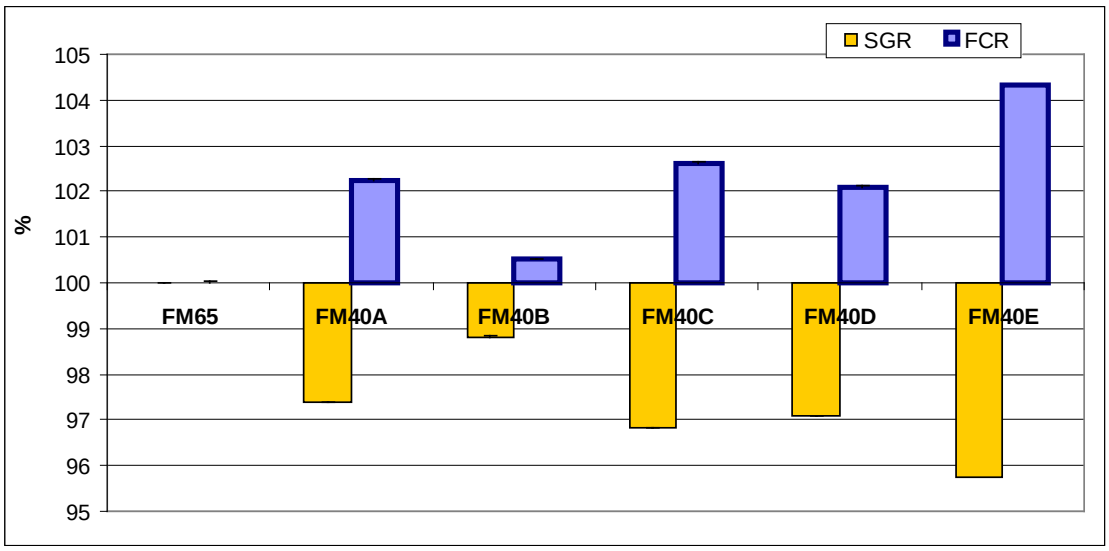

Fig. 5- Relative performance of the tested diets for European seabass.

Table 2-

Diet	FM65	FM40-A	FM 40-B	FM40-C	FM40-D	FM35-E
Survival (%)	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0
Initial weight (g)	102.10±1.45	100.66±1.29	99.60±5.36	99.38±2.00	100.63±5.57	101.40±2.48
Final weight (g)**	186.82±1.21	181.31±3.71	180.95±9.44	178.41±4.05	181±11.64	180.84±4.69
Weight gain (g)	84.72±1.45	80.65±2.94	81.35±4.23	79.03±2.05	80.374±6.10	79.45±3.59
SGR (%/d)/ind	0.81±0.02	0.78±0.02	0.80±0.01	0.78±0.0	0.78±0.01	0.77±0.03
Total feed/ind (g)	103.32±1.08	100.54±1.36	99.73±4.94	98.93±2.26	100.02±6.23	101.03±1.91
Feed intake (%ABW/d)*	0.95±0.0	0.95±0.0	0.95±0.0	0.95±0.0	0.95±0.0	0.95±0.0
FCR	1.22±0.03	1.25±0.03	1.23±0.02	1.25±0.01	1.25±0.02	1.27±0.05
HSI (%)	2.16±0.28	2.36±0.15	1.90±0.20	2.24±0.21	1.88±0.19	2.00±0.31
VSI (%)	8.41±0.68	8.36±0.08	8.17±1.20	8.64±0.62	7.63±0.82	8.77±1.25
FI (%)	21.09±0.09	20.06±0.95	20.77±1.66	20.37±0.57	20.73±0.72	20.90±0.56

* Feed intake expressed as percentage of average body weight per day

**Mean (± SD) of three replicates; values within the same row followed by different letters are significantly different ($P<0.05$)