TRIAL REPORT: SEA BREAM

Determine optimal levels of fishmeal/fish oil replacement with soy products (soybean meal, soybean oil and soy protein concentrate) in practical feeds for Gilthead sea bream (*Sparus aurata*)

Scientific team

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1 Objective

The results from the trials performed during 2007, indicated that none of the evaluated parameters was significantly affected by the different replacement levels (50% and 35% fishmeal protein) compared to the control (65% fishmeal protein). Growth and food conversion were not affected by the use of different soybean products and the histological study of the intestinal epithelium did not show any pathological sign due to the replacement.

The objective of the 2008 program for Gilthead seabream was focused on the 25% FM formulations (25% of dietary protein coming from FM) since they have currently the highest potential for being cost effective. This 25% FM formulation has a 17.5 % inclusion of S American FM. The exploration of the performance of further reducing fishmeal protein (down to 15% FM protein) was also included in this study.

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

Reference diet

1. **FM65**, high quality reference feed, 65% of dietary protein provided by FM; 95% of dietary fat provided by FM/FO.

Significant fishmeal/oil replacement: FM25 diets

FM25: marine fish feed with significant replacement of fishmeal/fishoil with soybean protein/fat: 25% of dietary protein is provided by FM and 50% of dietary protein is from soybean origin; 60 % of dietary fat provided by FM/FO;
The fishmeal/fish oil replacement by vegetable protein/fat is compensated with:

- palatability enhancers to correct for reduced feed intake in marine fish fed low fishmeal rations;
- essential amino acids (lysine, methionine) to maintain levels similar to the levels in the FM65 diet
- available phosphorous (under the form of mono calcium phosphate) to maintain levels well above minimum requirement of 0.5% available phosphorous

Three treatments were evaluating different proportions of SBM and SPC:

2. **FM25A**: maximizes on formula cost reduction by using high proportion of SBM.
3. **FM25B**: SBM/SPC1, evaluation of improving feed performance by increasing the contribution of SPC in the soybean protein supply (ratio SBM/SPC, 20/19.5%)
4. **FM25C**: SBM/SPC2 evaluation of improving feed performance by further increasing the contribution of SPC in the soybean protein supply (ratio SBM/SPC, 10/27%)

Extreme fishmeal/oil replacement: FM15 diets

FM15: marine fish feed with extreme replacement of fishmeal/fishoil with soybean proteins/fats: 15% of dietary protein is provided by FM and 55 % of dietary fat provided by FM/FO. Two treatments were formulated:

5. **FM15A**: maximising on formula cost reduction by using high proportion of SBM, supplemented with SPC- maximising soybean inclusion to provide 55% of dietary protein in combination with traditional vegetable protein concentrates (wheat/corn gluten); compensating the fishmeal/fish oil replacement with:
   - palatability enhancers to correct for reduced feed intake in marine fish fed low fishmeal rations;
   - essential aminoacids (lysine, methionine) to maintain levels similar to the levels in the FM65 diet
   - available phosphorous (under the form of mono calcium phosphate) to maintain minimum requirement of 0.5% available phosphorous

6. **FM15B**: in this treatment the same levels of fishmeal/fish oil were applied as in feed FM15A but the formulation was compensated on four fronts to maximize the chances of success with very low levels of FM:
   - Improve the essential amino acid balance to approach better the "ideal protein" (using pea protein and increasing the supplementation of lysine, methionine and threonine to approach better the AA profile of whole bream)

- Although the known minimum requirements for essential amino acids (EAA) are met in FM 15A (see table), we explored in FM15B the application of the ideal protein concept. The EAA balance in bream whole body was used to recalculate the levels of EAA required in a 45% protein diet. The levels of lysine, methionine and threonine were set according to the ideal protein concept using crystalline amino acids and blending different vegetable protein sources, which resulted in a higher supplementation of lysine and methionine, and the introduction of threonine in the diet compared to the FM15A diet. The other EAA levels were balanced as good as possible by adapting the mixture of vegetable protein concentrates (reducing the soybean protein supply to 50%, introducing pea protein concentrate, reducing corn gluten and eliminating wheat gluten).
- Increase the available phosphorous levels to get closer those of the FM65 diet
- Improve the feed palatability by increasing the level of palatability enhancers (palatability is well documented problem in marine fish fed low levels of FM)
- Working on the lipid digestibility by adding Easy Digest (lipid digestion/absorption is documented to be a problem in carnivorous fish fed high levels of vegetable protein)

Detailed formulation is presented in Table 1.

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 300 L, with well seawater of 23 ± 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 6-7 l/tank/min. Initial density was 1.30 Kg/m^3, with 50 individual per tank. The tanks were connected to a biofiltration unit of 2 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, *S. aurata* fingerlings, were obtained from a local hatchery. Trial duration was 11 weeks (2 weeks acclimation and 9 weeks 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 two 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.

Table 1: Feed formulations:

ASA 08 BREAM FORMULAS	FM65	FM25 A	FM25 B	FM25 C	FM15 A	FM15 B
S Am Prime Fishmeal (67%CP)	42,5%	16,4%	16,4%	16,4%	9,8%	9,8%
Fish Oil	15,7%	10,6%	10,7%	10,7%	10,8%	10,5%
SBM (Provasoy Cargill) (50%CP)	18,3%	36,2%	20,0%	10,0%	30,0%	30,0%
SPC (Danpro A Solea) (65%CP)		7,2%	19,5%	27,0%	15,4%	11,9%
Soya Oil		6,0%	6,0%	6,0%	6,0%	6,0%
Soybean lecithin		0,8%	0,8%	0,8%	1,0%	1,0%
Wheat Gluten (78%CP)	3,1%	5,4%	4,8%	4,4%	6,1%	
Corn Gluten (60%CP)	4,0%	8,0%	8,0%	8,0%	10,0%	6,3%
Pea Protein (78%CP)						11,6%
Wheat Flour	15,9%	7,0%	11,5%	14,2%	7,9%	8,1%
Vit-Min Premix	0,50%	0,50%	0,50%	0,50%	0,50%	0,50%
Mono Ca Phosphate		0,60%	0,60%	0,60%	0,80%	1,63%
L-Lysine (78%)		0,30%	0,30%	0,30%	0,45%	0,53%
DL-Methionine (99%)		0,15%	0,15%	0,15%	0,20%	0,37%
Threonine (98%)						0,24%
Palatability enhancer (Easy Appetite, INVE Aquaculture)		0,83%	0,83%	0,83%	1,00%	1,00%
Digestibility enhancer (Easy Digest, INVE Aquaculture)						0,50%
SUM	100,0%	100,0%	100,0%	100,0%	100,0%	100,0%
Formulated values						
Crude Protein (%)	45,0	45,0	45,0	45,0	45,0	45,0
Crude Fat after Hydrolysis (%)	20,0	20,0	20,0	20,0	20,0	20,0
Crude Fibre (%)	1,0	1,7	1,9	2,0	2,0	1,8
Crude Ash (%)	8,3	6,9	6,8	6,7	6,4	7,2
Protein from fishmeal (% of tot protein)	65%	25%	25%	25%	15%	15%
Protein from Soya (% of tot protein)	20%	50%	50%	50%	55%	50%
Protein from Wheat Gluten (% of tot protein)	5%	9%	8%	8%	11%	
Protein from Corn (% of tot protein)	6%	12%	12%	12%	15%	9%
Protein from Pea (% of tot protein)						20%
Vit E (ppm)	250	250	250	250	250	250
Vit C (ppm)	250	250	250	250	250	250
Calcium (%)	1,74	0,95	0,95	0,95	0,75	0,88
Phosphorus (%)	1,17	0,97	0,97	0,97	0,92	1,18
Available Phosphorous (%)	0,65	0,50	0,50	0,50	0,46	0,65
LYS (%)	2,67	2,62	2,63	2,63	2,61	3,10
MET (%)	0,96	1,01	1,01	1,01	1,04	1,15
THR (%)	1,74	1,65	1,66	1,66	1,62	1,92
ARG (%)	2,58	2,67	2,68	2,69	2,65	3,01
PHE (%)	1,95	2,16	2,15	2,15	2,21	2,05
HIS (%)	0,98	1,04	1,04	1,04	1,05	1,07
ILE (%)	1,90	1,90	1,90	1,90	1,89	1,98
LEU (%)	3,45	3,71	3,70	3,70	3,82	3,70
VAL (%)	2,18	2,07	2,08	2,08	2,04	2,12

- Stocking and sampling

For all the handling of the animals, phenoxyethanol has been used as anaesthetic. Before stocking, the fish were sampled (weight in water) to determine size distribution. Stocking has been done sequentially. Diets were assigned to the tanks at random.

Sampling happened every two weeks; for the sampling, all the fish/tank have been weighed in groups and carefully observed to check the health status of the animal. From this sampling, average weight and survival have been determined and feed ration adjusted accordingly every two weeks.

At final sampling fish have been weighed individually and 5 individuals per tank (15 per treatment) have been picked randomly for dissection to determine HSI/VSI index and filet collection for further analyses; from these 5 fish per tank, 3 of them were used for histological sampling (intestine dissection; 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 (one side filet weight*100/total weight)
- Gut histology

3 Results

Growth results

Seabream juveniles have been during 11 weeks in the experimental system; the first two weeks have been considered as acclimation period and experimental feeds have been provided during the next 9 weeks. The data presented are the average of three replicates per treatment.

Control treatment, FM65, had the highest growth during the trial (SGR, 2.55 %/day) closely followed by the treatment FM15B (SGR 2.47). ANOVA analysis showed significant difference among treatments for SGR, FCR, Total feed intake and feed intake expressed as % of average body weight confirming the difference in performance(see Table 2) of the different diets.

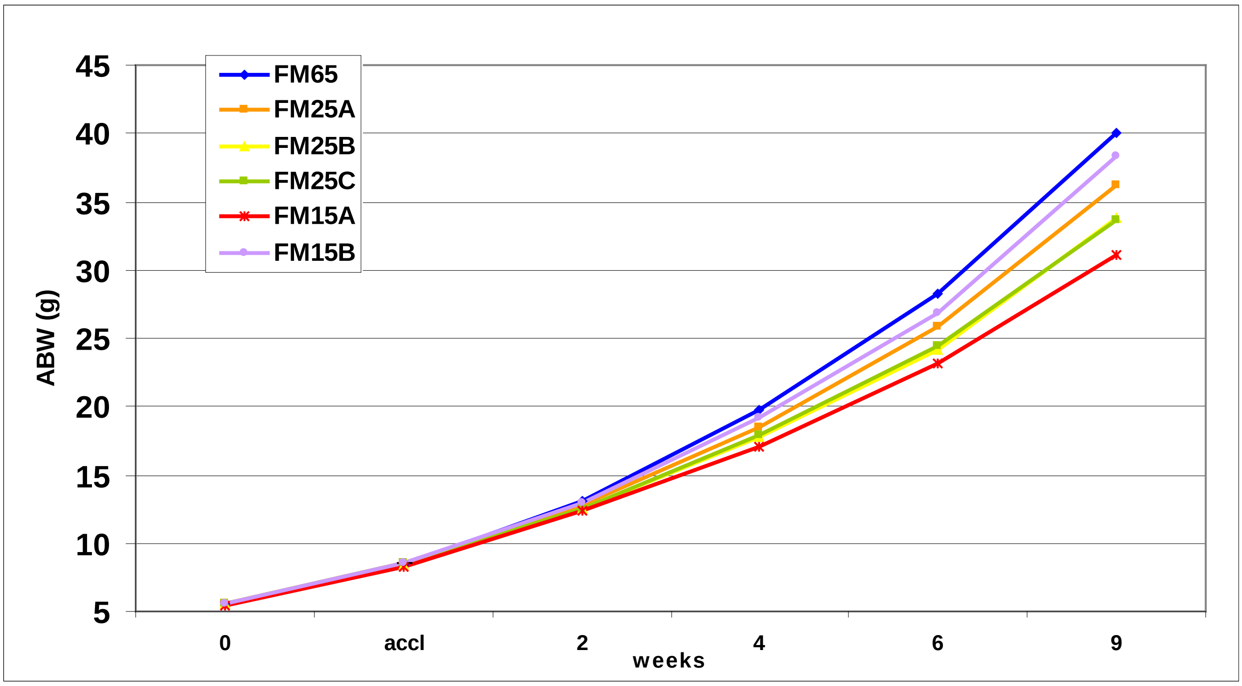

Fig. 1- Growth curve of seabream fed during 9 weeks the experimental diets (first two weeks were acclimation period)

There was no significant mortality during the trial. In general, feeding behaviour of the fish was very good albeit significant differences (*P>0.05*) were observed in total feed intake per individual and feed intake expressed as percentage of the average body weight (Table 2). Accordingly, final weight and total weight gain, showed also significant differences among diet. Treatment FM65 performed best, with a SGR of 2.55 %/day, followed by diet FM15B with 2.47 %/day. Diets FM25A, FM25B and FM25C had 2.38, 2.27 and 2.26 %/day respectively; FM15A showed the lowest SGR value with 2.16%/day.

The FCR values obtained are in the range of 1.04 and 1.24. Significant differences have been found among the different diets (*P>0.05)*. Diet FM65 showed the best FCR values (1.04±0.06) closely followed by FM15B (1.05±0.03). Diet FM15A presented the highest value, 1.24. (Table 2).

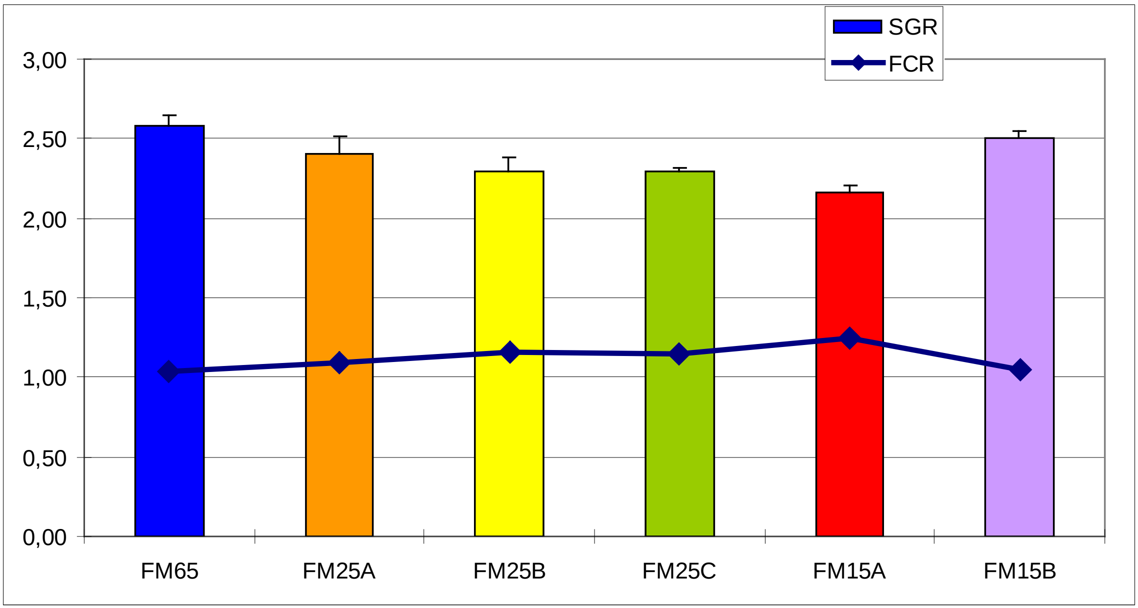

Fig. 2- SGR and FCR of seabream fed experimental diets during 9 weeks.

Concerning the HSI, VSI and filet index, no significant differences were found among the different diets. All diets yielded similar values; however, if we compare the HSI value of the FM65 diet with diets FM25A, FM15A and FM15B, the three of them showed a lower HSI value (percentage wise 8.55, 2.89 and 6.51% respectively). With regard to VSI, following the same comparison with control diet, FM65, only two diets had lower VSI values: FM25A and FM15B, 4.82 and 2.39 % respectively.

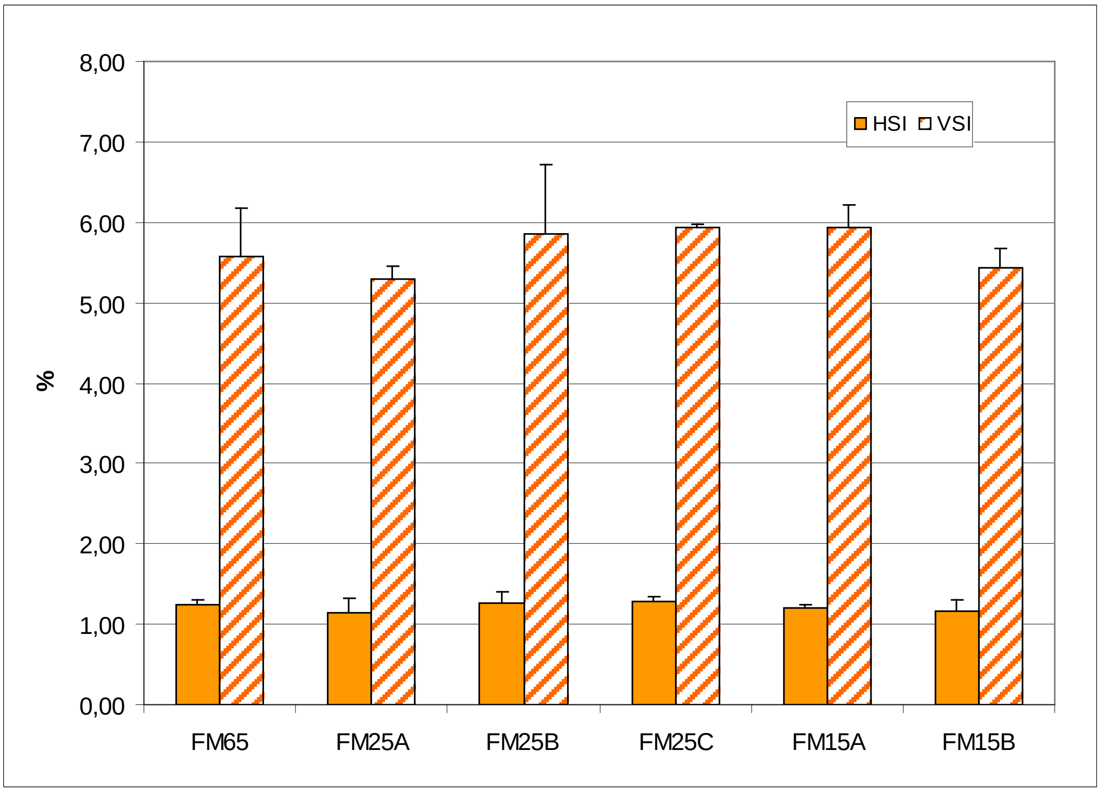

Fig. 3- HSI and VSI of seabream fed experimental diets during 9 weeks. Represented values are the mean and standard deviation of 15 replicates.

Filet analyses:

With regard to proximal composition of the filet, the results are presented in Table 3. No significant difference was found among the treatments in filet composition (*p<0.05*). Interesting to see that for Crude fat, diet FM15B has the lowest value (8.13 % ww), 25 % lower than the FM65 (10.73 %ww).

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

Diet	FM65	FM25A	FM25B	FM25C	FM15A	FM15B
Dry matter	29.53±0.25	29.43±0.50	30.77±1.80	31.07±0.93	29.53±0.38	29.23±0.25
Crude Protein	19.57±0.35	19.60±0.35	19.43±0.21	19.47±0.25	19.37±0.21	19.73±0.55
Crude fat	10.73±2.87	9.67±0.61	11.17±1.57	9.90±1.11	8.50±0.30	8.13±0.40
Crude ash	1.60±0.16	1.59±0.14	1.60±0.24	1.54±0.10	1.58±0.06	1.59±0.06

Histological analyses

No significant alterations have been found in any of the examined samples. Most of the intestine samples show a normal aspect and only one single sample (7C) showed some slight changes from

the normal structure. These changes are basically a moderate increase of the presence of inflammatory cells (most of them lymphocytes), diffusely distributed in the intestinal submucosa of the posterior intestine but also in the mucosa. However, samples 7A and 7B do not present any deviations from the normal structure. The other parts of the intestine do not present any significant changes as well. No signs of infection or presence of intestinal parasites have been noticed in any of the samples, including sample 7C.
Images of this histological study are included in a separate document.

4 Conclusion

Compared with the results of 2007 seabream program (where FM replacement was evaluated to the level of 50% and 35% FM protein), these 2008 results show a significant difference of the FM65 diet with the FM25 diets. The relative difference for the replacement for the diets FM25A, FM25B and FM25C, with increasing SPC inclusion (SBM/SPC ratio, 36.2/7.2, 20.0/19.5 and 10/27 % respectively) was lower in the diet FM25A (lowest inclusion of SPC), i.e. 7% lower SGR and 5.73% higher FCR.

Deepest replacement diets, FM15A and FM15B presented very different performance. FM15A, with higher inclusion of wheat gluten and corn gluten, had a 15.56% lower SGR and 19.49% higher FCR compared with FM65 (Fig.4). Interesting to check that diet FM15B, with the accent on the amino acid profiling, available phosphorous and palatability/digestibility enhancers did not show significant different performance compared to FM65 control, i.e. 3.35% lower growth and 1.11% higher FCR than the FM65 diet. Also interesting is the fact that FM15B diet had the lowest fat content in the filet (25% lower than the control diet, FM65).

From the results of the present trial, it seems that the use of low fishmeal inclusion (i.e. 15% of the dietary protein originating from fishmeal, diet FM15B) in practical diets for Gilthead seabream, gives no significant difference in growth and food conversion compared with standard commercial diet (FM65, 65% of the protein coming from FM), if the formula is compensated with a proper nutritional balance and adequate palatability/digestibility enhancers.

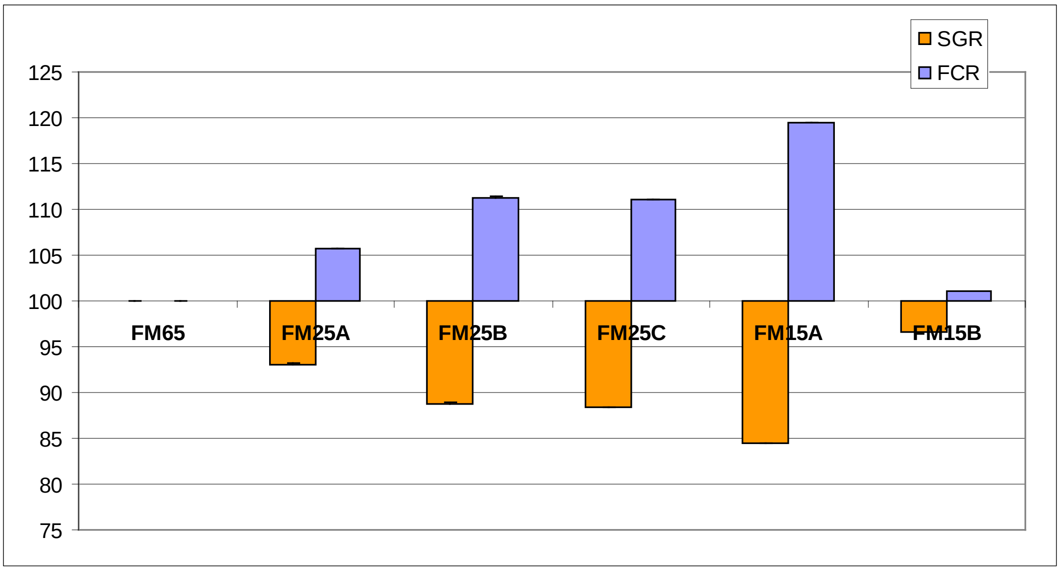

Fig.4- Relative performance of the different replacement diets compared with the FM65

Table 2-

Diet	FM65	FM25A	FM 25B	FM25C	FM15A	FM15B
Survival (%)	96.00±0.0	97.33±2.31	94.67±1.15	98.67±1.15	96.00±2.00	99.33±1.15
Initial weight (g)	8.43±0.09	8.48±0.02	8.44±0.05	8.48±0.22	8.33±0.07	8.49±0.18
Final weight (g)**	40.09±1.70 [a]	36.19±2.30 [bc]	33.74±1.90 [cd]	33.60±0.45 [cd]	31.07±0.88 [d]	38.27±0.99 [ab]
Weight gain (g)	31.65±1.74 [a]	27.71±2.32 [bc]	25.29±1.88 [cd]	25.13±0.36 [cd]	22.74±0.87 [d]	29.78±1.07 [ab]
SGR (%/d)/ind	2.55±0.08 [a]	2.38±0.11 [bc]	2.27±0.09 [c]	2.26±0.03 [c]	2.16±0.04 [d]	2.47±0.07 [ab]
Total feed/ind (g)	32.90±0.28 [a]	30.41±0.89 [c]	29.23±0.47 [d]	29.06±0.37 [de]	28.27±0.33 [e]	31.33±0.23 [b]
Feed intake (%ABW/d)*	2.22±0.07 [a]	2.23±0.05 [a]	2.27±0.07 [ab]	2.26±0.01 [a]	2.35±0.03 [b]	2.20±0.03 [a]
FCR	1.04±0.06 [a]	1.10±0.06 [ab]	1.16±0.07 [bc]	1.16±0.02 [b]	1.24±0.03 [c]	1.05±0.03 [a]
HSI (%)	1.24±0.07	1.14±0.19	1.27±0.13	1.29±0.05	1.21±0.04	1.16±0.14
VSI (%)	5.57±0.61	5.30±0.15	5.86±0.86	5.93±0.05	5.94±0.27	5.44±0.24
FI (%)	19.02±0.49	19.15±0.09	18.96±0.24	19.19±0.13	18.63±0.37	18.96±0.48

* 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*)