

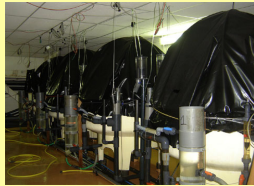
Proteomic approach to investigate alterations, within physiological limits, in serum protein of sea bass (*Dicentrarchus labrax*)



Jean Luc Coeurdacier,
IFREMER Laboratoire Halieutique méditerranéen et Tropical, Rue Jean Monnet, BP:171 34203 Sète France
jlcoeurd@ifremer.fr tel (33)4 99 57 32 48



1/ Purpose



Total Serum Protein (TPS) level can discriminate high modifications in rearing conditions, but it cannot detect low alterations, within normal limits (welfare) like high stocking density. In order to discriminate those situations, serum protein panels were investigated by proteomic approach: 2D electrophoresis, LC-MS-MS⁽¹⁾ and Seldi-tof ⁽²⁾



2/ TSP discrimination limits

The effects of a high stocking density (100kg/m³) were monitored on sea bass with all water parameters maintained at non limiting levels.

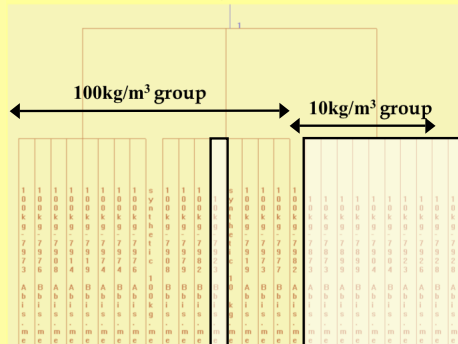
The global results were:

Alteration of swimming behaviour, feed intake and growth rate Respiratory activity higher for the 100 kg/m³ density No difference was found in the feed conversion ratio and fin damages.

No difference on blood analysis and no conclusive difference in Total Serum Protein

4/ Statistical analysis

Heuristic clustering plot (%vol)



Intra class CR (% volume)

0.98 > C.R. > 0.93 in 10kg/m³
0.95 > C.R. > 0.84 in 100kg/m³

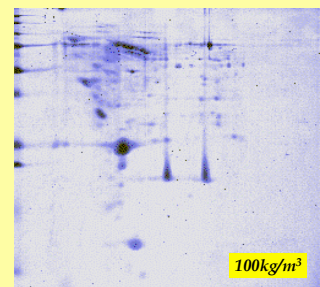
Inter class Kolmogorof Smirnof Different (P<0.05)

11 gels in 10kg/m³
15 gels in 100kg/m³

Heuristic clustering performed on all gels shared them into 3 classes. One including gels from 10kg/m³ and 2 including 100kg/m³. Only one 10kg/m³ gel is not in the right class.

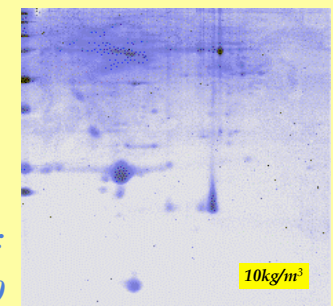
3/ 2D gels and spot analysis

15 sera from both group analysed on 18-cm pH NL gd strips pH 3-10; Coomassie blue stained (BIO-RAD -Protean Dodecca cell)



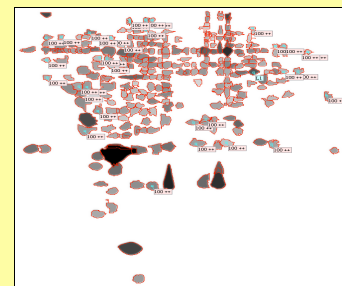
Spots counted on gels

100kg/m³ :
360-450

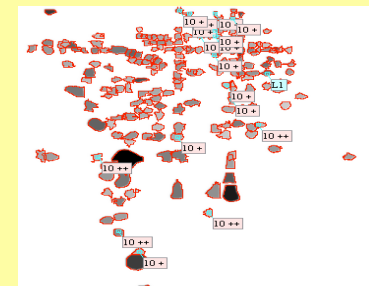


10kg/m³ :
226-370

Synthetic gels obtained with 6/11 gels for 10 kg/m³ and 8/15 with 100 kg/ml³ ImageMaster 2D platinum 6.0 GeneBio.



90/280 spots (labelled) present only in 100kg/m³



17/204 spots (labelled) present only in 10kg/m³

5/ LC-MS-MS Spot identification

20 spots significantly different between 100kg/m³ and 10kg/m³ (Kolmogorov-Smirnov test)

were selected and excised for analysis on LC MS-MS. Four spots (11, 12, 21, 23 Arrow) were of interest

The 4 spots were identified

- Inter-alpha (Globulin) inhibitor H3

-C3 complement and FBP32

-Warm temperature acclimatisation related protein

Proteins probably involved in inflammation



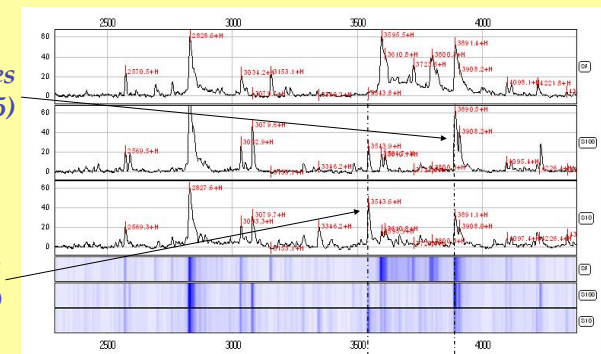
6/ SELDI-TOF Ciphergen's protein chip system



- It works on whole serum sample
- It permits to discriminate pick alteration in a few hours
- Pre-selection of proteins (hydro/lipophyl...) by the choice of chip surface
- Intensity level optimises pick visualisation
- But:
- Available only for small molecules (50Kda)
- It does not allowed identification

Pick 3890 increases in 100kg/m³ (p<0.05)

Pick 3543 increases in 10kg/m³ (p<0.05)



7 Conclusion

2D gel electrophoresis show difference between serum proteome of sea bass reared at 100kg/m³ and 10kg/m³ stocking density
4 proteins probably involved in inflammation were identified at 100kg/m³ by LC MS-MS
SELDITOF permits to show difference in protein panel between the 100kg/m³ and 10kg/m³ rearing density

Proteomic analysis can discriminate modifications within normal limits in rearing conditions

[1] LC-MS-MS: Liquid chromatography- mass spectrometry (Tandem)

[2]SELDI-TOF : surface enhanced laser desorption and ionization - time of flight

[3] Sammouth et al 2008. Aquacultural engineering 40(2) 72-78