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

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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 and Seldi-tof.

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

- Intra class CR (% volume) 0.98>C.R.>0.93 in 10 kg/m³
- Inter class Kolmogorov Smirnoff Different (P<0.05) 11 gels in 10 kg/m³, 15 gels in 100 kg/m³

5/ LC-MS-MS Spot identification

20 spots significantly different between 100 kg/m³ and 10 kg/m³ (Kolmogorov-Smirnoff test) were selected and excised for analysis on LC-MS-MS. Four spots (11, 12, 21, 23 Arrow) were of interest.

- 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
- Available only for small molecules (50Kda)
- It does not allowed identification

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

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

7 Conclusion

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

Proteomic analysis can discriminate modifications within normal limits in rearing conditions.

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[1] LC-MS-MS: Liquid chromatography-mass spectrometry (Tandem)
[2] SELDI-TOF: Surface enhanced laser desorption and ionization – time of flight

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