



EFFECTS OF ERGOSAN ON THE INNATE IMMUNOSYSTEM AND ON STRESS TOLERANCE ON RAINBOW TROUT *Oncorhynchus mykiss*

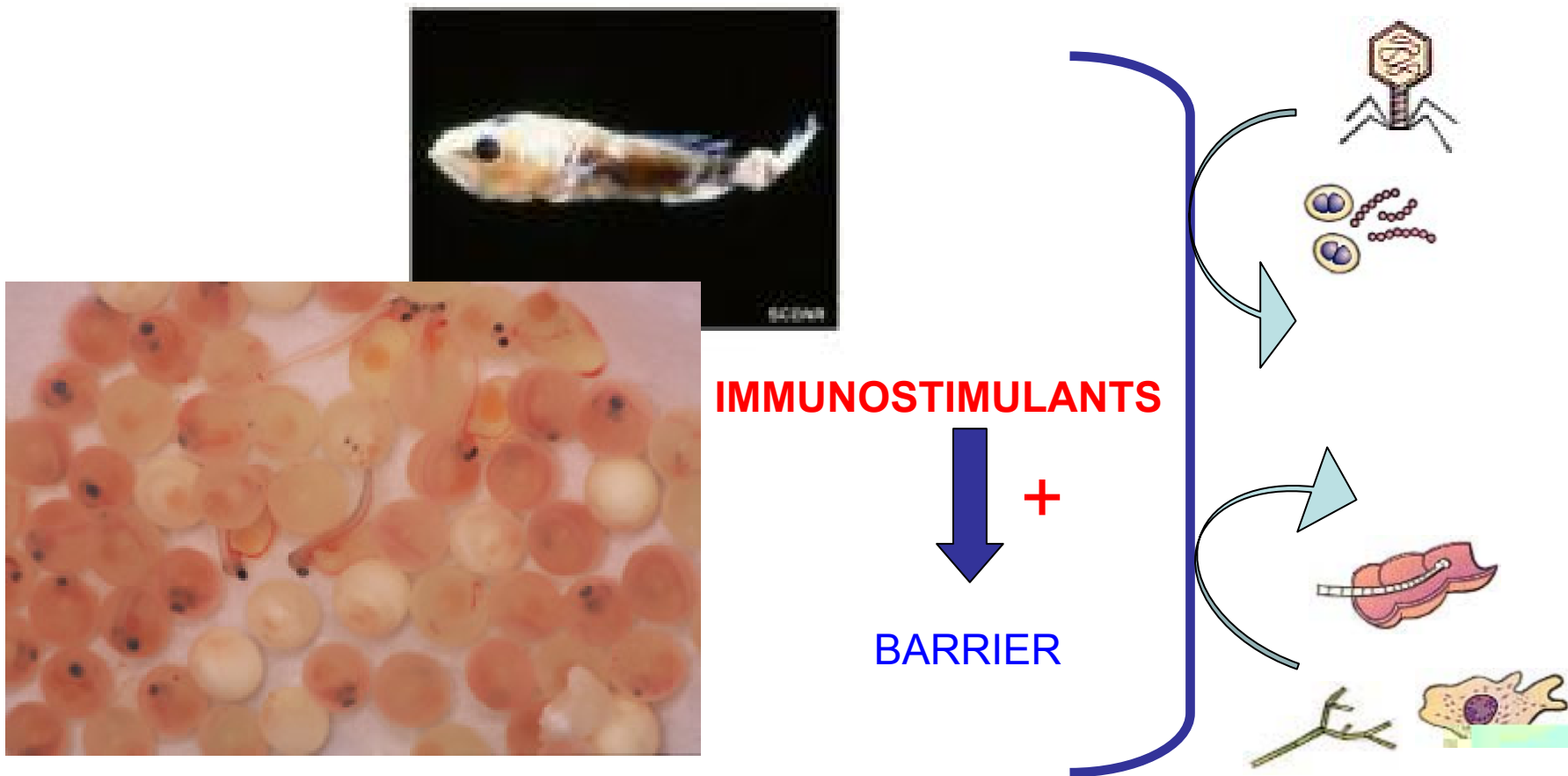
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THE IMMUNOMODULATION OF LARVAL FISH HAS BEEN PROPOSED AS A POTENTIAL METHOD FOR IMPROVING LARVAL SURVIVAL BY INCREASING THE INNATE RESPONSES OF THE DEVELOPING ANIMALS, UNTIL ITS ADAPTIVE IMMUNE RESPONSE IS SUFFICIENTLY DEVELOPED TO MOUNT AN EFFECTIVE RESPONSE TO THE PATHOGEN

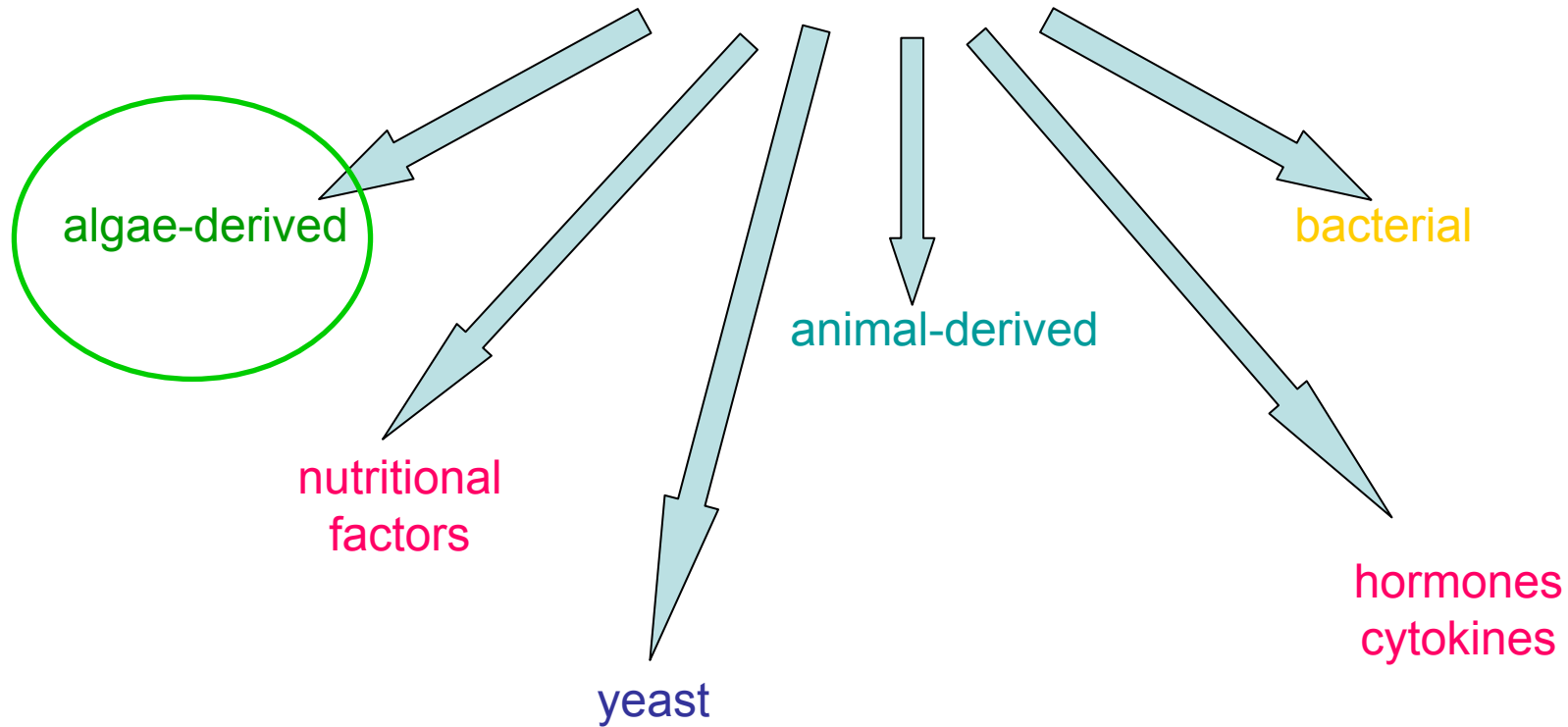
THE USE OF IMMUNOSTIMULANTS, AS DIETARY SUPPLEMENTS, CAN IMPROVE THE INNATE DEFENCE OF JUVENILES FISH PROVIDING RESISTANCE TO PATHOGENS DURING PERIODS OF HIGH STRESS, SUCH AS GRADING, MANIPULATION AND VACCINATION



WHAT IS AN IMMUNOSTIMULANT?

“An immunostimulant is a naturally occurring compound that modulates the immune system by increasing the host’s resistance against diseases that in most circumstances are caused by pathogens”

IMMUNOSTIMULANTS

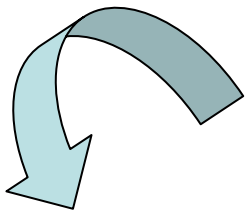




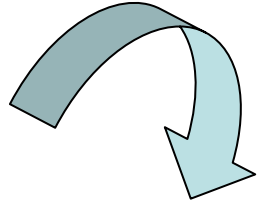
ERGOSAN (Schering-Plough Aquaculture, UK) is a complementary feedingstuff, made from a seaweed-based meal rich in alginates and polysaccharides. In particular it is composed of extracts from *Laminaria digitata* and *Ascophyllum nodosum*.

It has been demonstrated that:

- A single dose of 1 mg of Ergosan significantly augmented the proportion of neutrophils, increased the degree of phagocytosis, respiratory burst activity and expression of interleukin-1b (IL-1b), interleukin-8 (IL-8) and tumour necrosis factor-alpha (TNF-a) in peritoneal leucocytes at 1 day post-injection in trout (Peddie S *et al.*,2002)
- An oral administration of Ergosan in sea bass, up-regulates the immune response (Bagni M *et al.*, 2005)

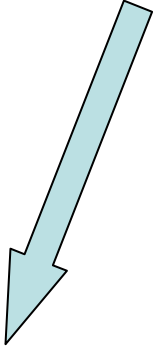


IMMUNOSYSTEM

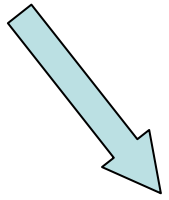


INNATE IMMUNITY

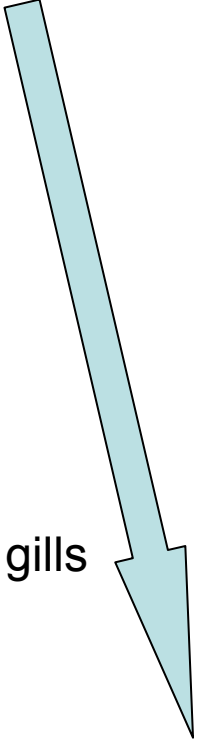
ADAPTIVE IMMUNITY



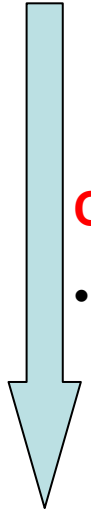
- PHISIC BARRIERS**
- FISH SCALES
 - EPIDERMIS
 - MUCUS of skin and gills



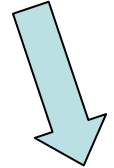
- CHEMICAL BARRIERS**
(ILs, TNF, IFN)



- BIOLOGICAL BARRIERS**
- MACROPHAGES/MONOCYTES
 - LEUCOCITE
 - NATURAL KILLER



- HUMORAL**
- ANTIBODY
 - LYMPHOCYTES B



- CELLS MEDIATED**
- LYMPHOCYTES T

IMMUNITY

INNATE

ADAPTIVE

CELLS:	MACROPHAGES/MONOCYTES, LEUCOCITE, NATURAL KILLER	T-, B-CELLS
TISSUE	LIVER, SPLEEN PRONEPHROS	LYMPHOID
REGULATORS	CYTOKINES	CYTOKINES
KINETICS	FAST (0-96h)	SLOW (after 96h)

•INNATE IMMUNE MECHANISMS ACT AS A FIRST LINE OF DEFENCE AGAINST INFECTION

•THIS NATURAL RESISTANCE IS NORMALLY EFFECTIVE ENOUGH TO PROTECT FISH FROM INFECTIOUS DISEASES UNTIL SPECIFIC IMMUNE RESPONSES ARE BEING ESTABLISHED

•WHILST A LARGE RANGE OF CELL TYPES AND MEDIATORS ARE INVOLVED IN INFLAMMATORY EVENTS, THIS WORK WILL FOCUS ON A GROUP OF MOLECULES THAT INITIATE AND REGULATE THESE PROCESSES, THE **CYTOKINES**

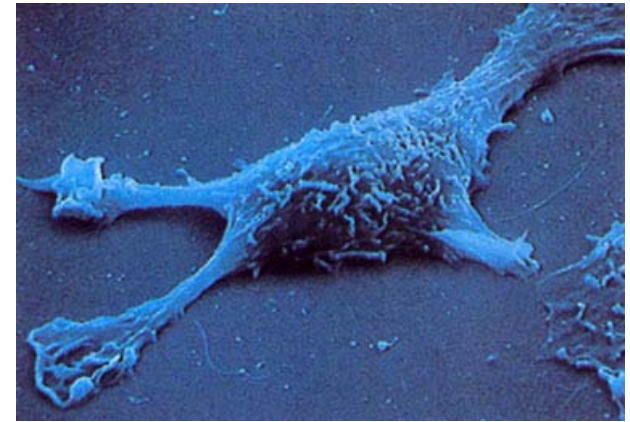
INTERLEUKINE 1



- Interleukin-1 β is one of the early response pro-inflammatory cytokines inducing a cascade of effects leading to inflammation.
- Many of these effects are mediated indirectly through up- or down regulation of other cytokines (i.e. IL-8).
- IL-1 is predominantly synthesised in monocytes but also by activated macrophages of different source, endothelial cells, fibroblasts, hepatocytes and many other cells type.



INTERLEUKINE-8



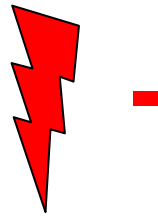
- Interleukin 8 was the first described among the chemokines
- Chemokines are a group of small secreted cytokines that controls the traffic of immune cells by leukocyte chemotaxis
- IL-8 can attract neutrophils, T lymphocytes and basophils to the inflammation site
- IL-8 is produced by a variety of cell-types including macrophages, monocytes, neutrophils, endothelial and epithelial cells

TUMOUR NECROSIS FACTOR α



- Tumour necrosis factor plays a crucial role in host immune responses and inflammatory processes
- TNF α is a type II transmembrane protein or glycoprotein, that is cleaved within the extracellular C-terminal domain to release biologically active mature peptide

STRESS

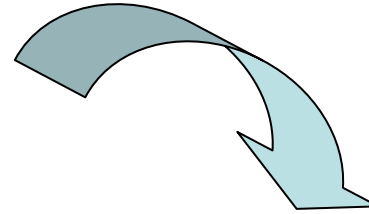
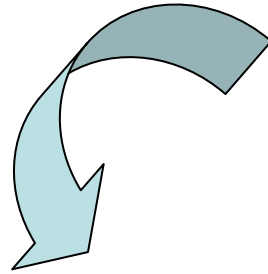


IMMUNOSYSTEM

STRESS

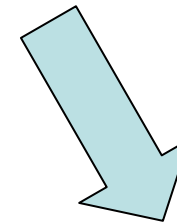
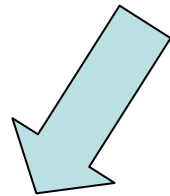


FISH



PHYSIOLOGICAL
RESPONSE

CELLULAR
RESPONSE



**CORTISOL
RELEASE**

**HSP70
SYNTHESIS**

PHYSIOLOGICAL STRESS RESPONSE

- Physiological stress response is characterized by an increase in stress hormones and consequent changes that help maintain the animal normal state
- It involves the activation of the hypothalamic-pituitary-interrenal axis that lead to cortisol release
- High cortisol levels are noxious to the immune system because they can inhibit the inflammatory response and antibody production

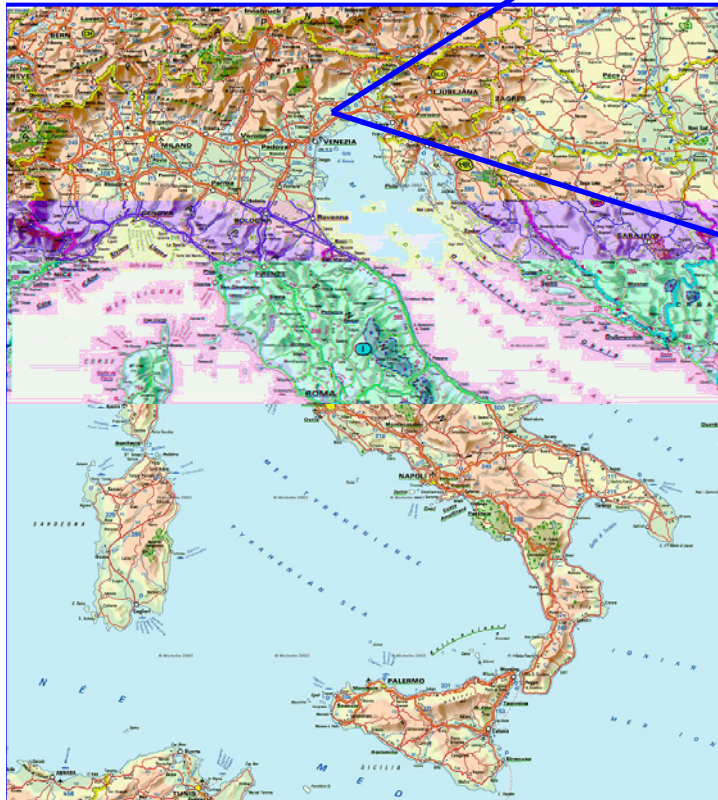
CELLULAR STRESS RESPONSE

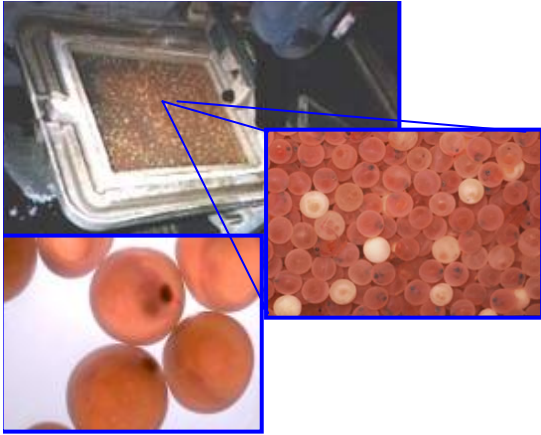
- Cellular stress response is the change in the concentration of HSP proteins (HSPs)
- HSPs are a family of highly conserved cellular proteins
- HSP 70 acts as a molecular chaperone, mediates the repair and degradation of altered or denatured proteins and prevents protein aggregates under physical stress

AIM

- **Verify the rearing condition tolerance improvement on fish fed on Ergosan, by monitoring body weight, cortisol plasma levels and liver Hsp70 gene expression**
- **Verify the heat stress tolerance improvement on fish fed on Ergosan by monitoring cortisol plasma levels and liver Hsp70 gene expression**
- **Verify the innate immune response improvement after heat stress on fish fed on Ergosan by monitoring liver cytokines genes expression**

“Le Gru” fishfarm, Udine





TREATMENTS

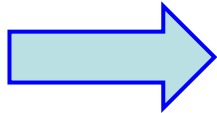
60000 rainbow trout embryos were divided in 6 groups each of 10.000 specimens:

- 1) commercial diet + Ergosan
- 2) commercial diet + Ergosan
- 3) commercial diet + Ergosan
- 4) commercial diet
- 5) commercial diet
- 6) commercial diet

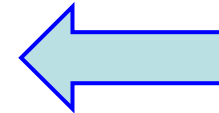
**ERGOSAN TREATED
GROUPS**

CONTROL GROUPS

**ERGOSAN
TREATED
GROUPS**



**CONTROL
GROUPS**



- **TANKS:** 6x1x0,8 m with a flow rate of 1500-2000 ml min⁻¹
- **O₂ CONDITION:** 8 ppm
- **TEMPERATURE:** 12 °C

First ERGOSAN administration started in juveniles 10 days from the beginning of first solid feeding

ERGOSAN ADMINISTRATION PROTOCOL AND SAMPLING

30 DAYS WITH ERGOSAN ← **1st SAMPLING (DAY 40 p.h.)**

15 DAYS WITH COMMERCIAL FEED

10 DAYS WITH ERGOSAN ← **2nd SAMPLING (DAY 65 p.h.)**

20 DAYS WITH COMMERCIAL FEED

10 DAYS WITH ERGOSAN ← **3rd SAMPLING (DAY 95 p.h.)**

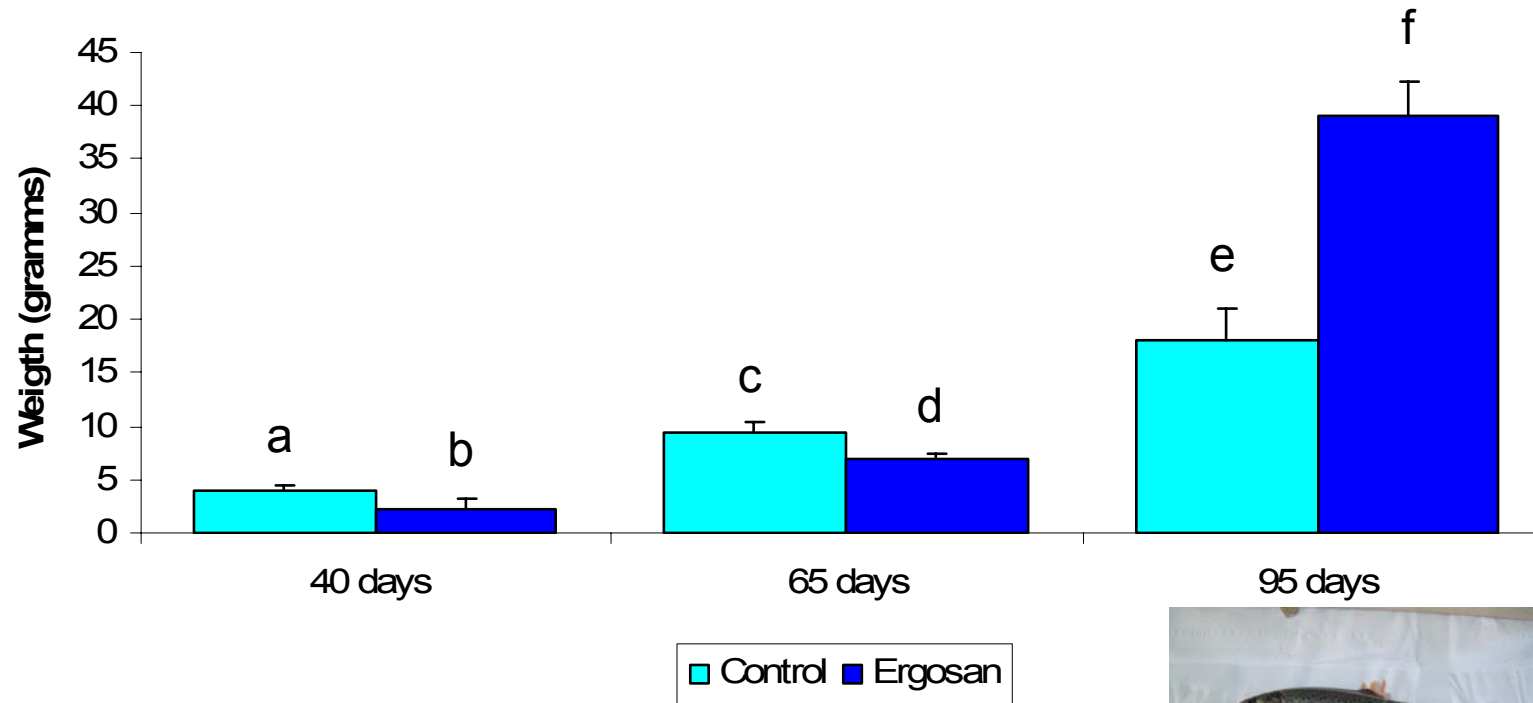
The controls fish were only fed on commercial diet and were sampled at the same time of the treated fish

RESULTS

EFFECTS OF **ERGOSAN** ON REARING CONDITION TOLERANCE:

- **GROWTH**
- **CELLULAR RESPONSE**
- **PHYSIOLOGICAL RESPONSE**

Body weight:

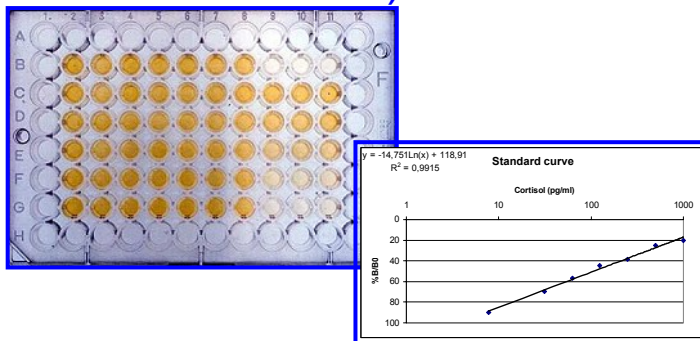


A dramatic increase of body weight was observed during the last sampling in animals treated with Ergosan

Molecular studies

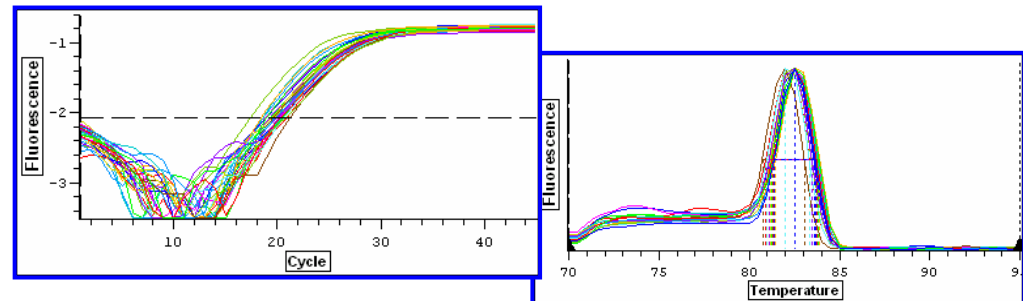
CORTISOL PLASMA LEVELS

The evaluation of cortisol levels in the plasma was performed by EIA using the Cortisol EIA kit (Cayman Chemical)

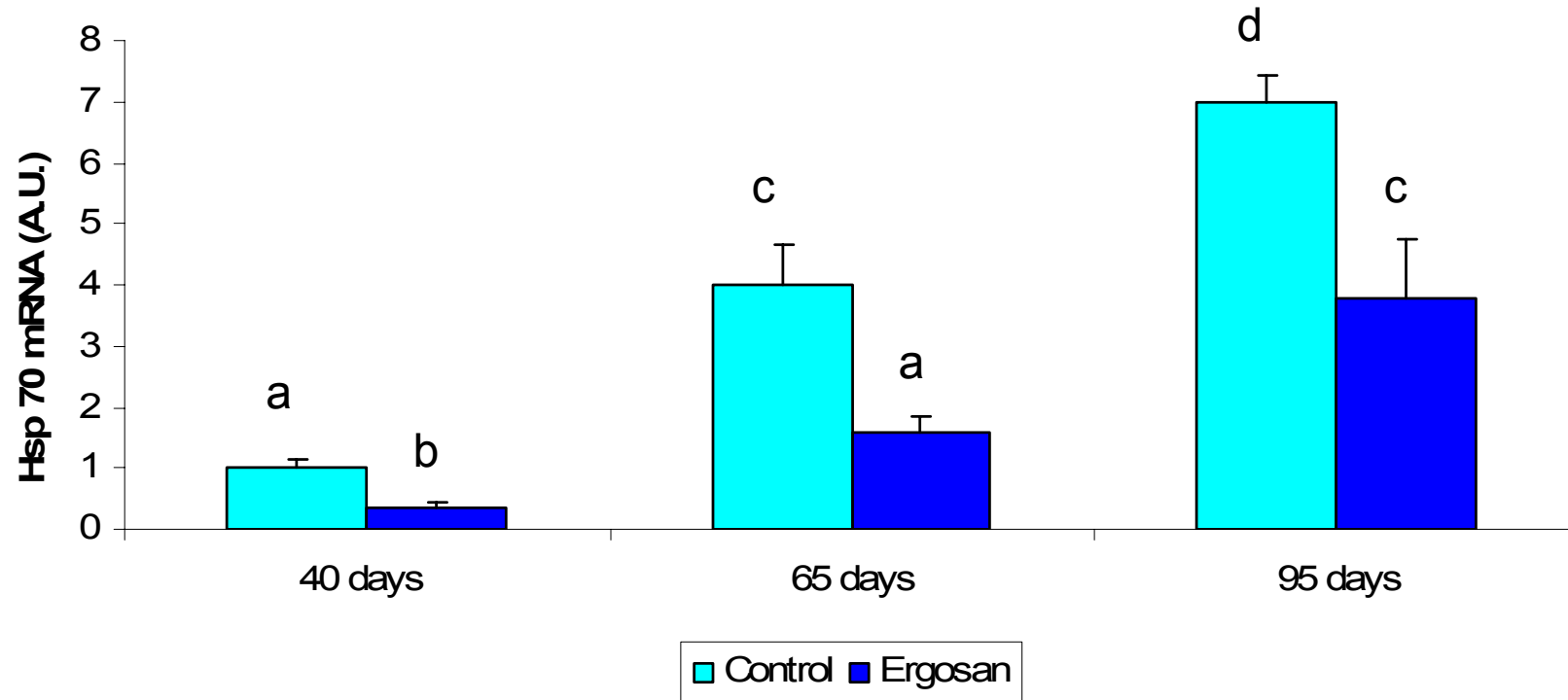
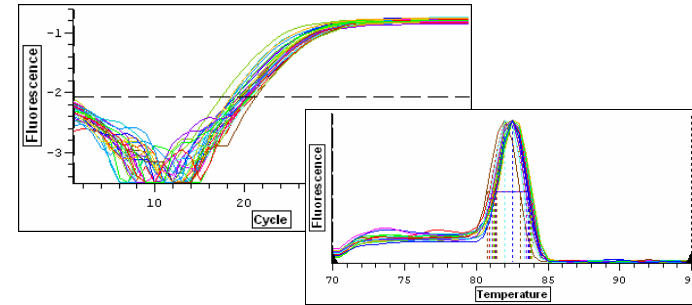


GENES EXPRESSION

IL-1, IL-8, TNF α Hsp 70 genes expression in trout liver was monitored by real time PCR using Acidic Ribosomal Phosphoprotein P0 (ARP) as internal standard.

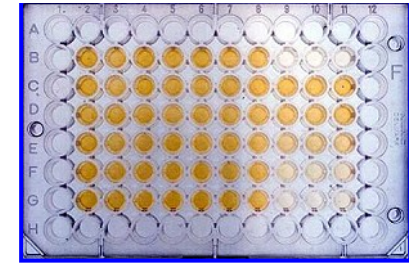
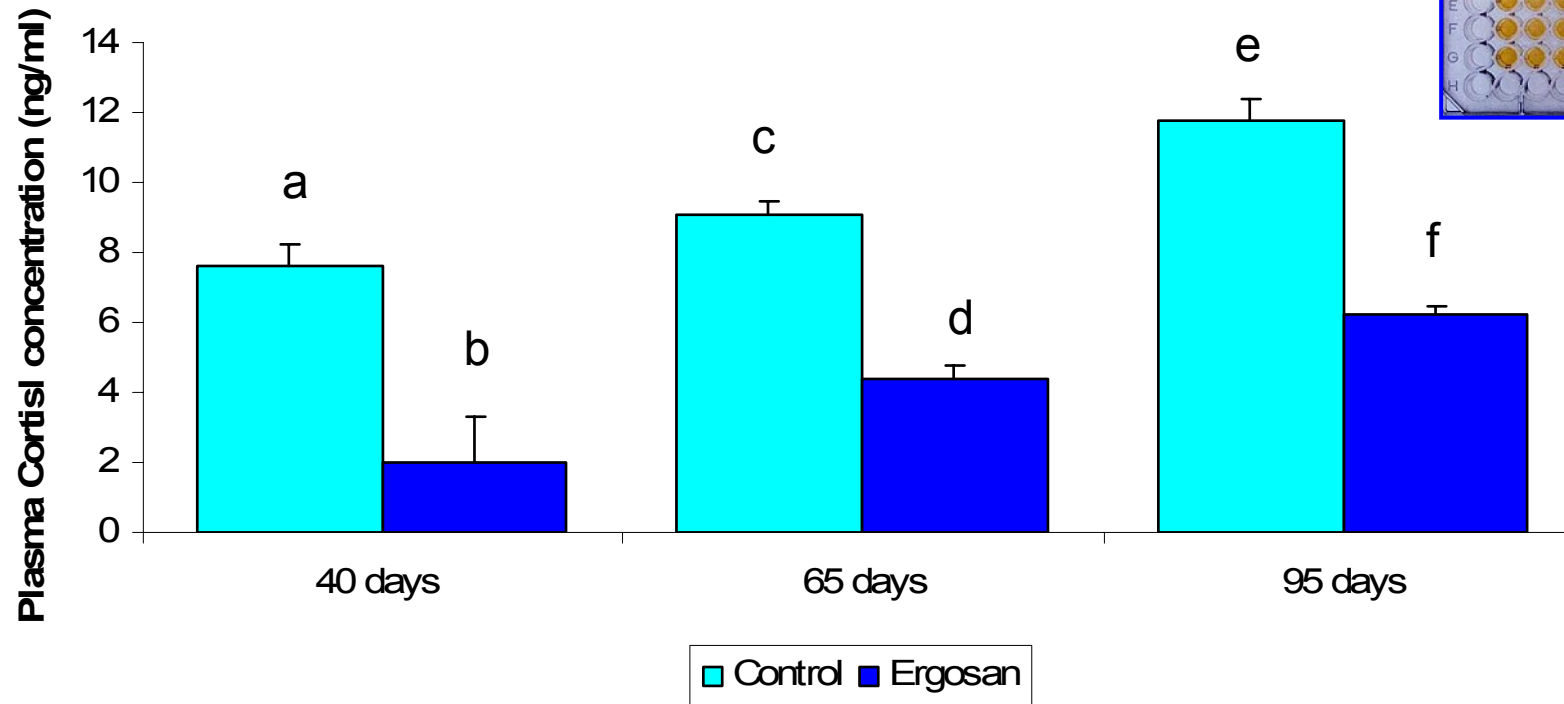


Cellular response:



The Real Time PCR results showed lower levels of Hsp70 gene expression in fish fed on Ergosan during all the treatment.

Physiological response:



The results demonstrated that the farming conditions during the time become more and more stressful for trout juveniles. Ergosan treatment induced a significant decrease of cortisol plasma levels during all the experimental times

These results demonstrated a positive role of Ergosan on trout juveniles welfare, having these fish lower levels of hepatic Hsp70 gene expression, lower plasma cortisol levels and higher body weight

EFFECTS OF ERGOSAN ON HEAT SHOCK STRESS TOLERANCE:

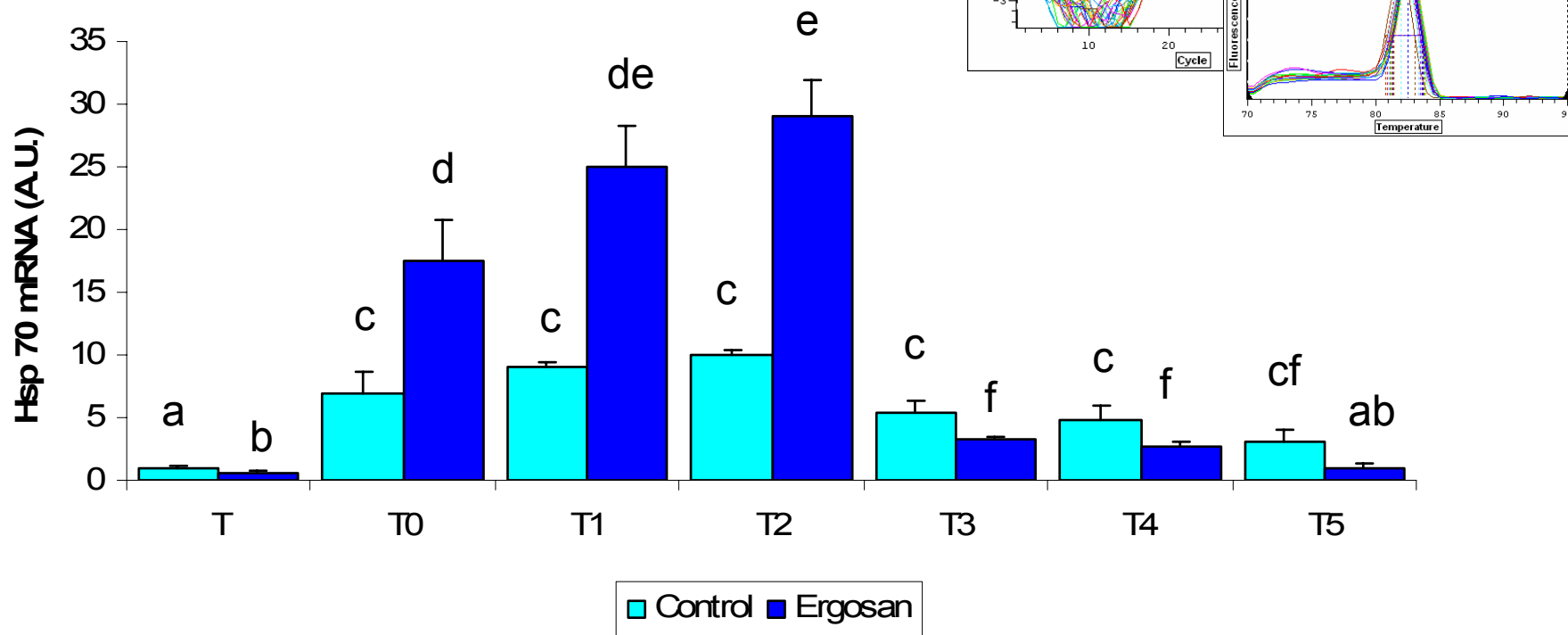
- CELLULAR RESPONSE**
- PHYSIOLOGICAL RESPONSE**
- INNATE IMMUNE RESPONSE**

HEAT SHOCK CONDITIONS

- From 12°C to 18°C, for 30 min
- After this time the temperature was shifted to 12°C time zero (T0)
- A time course to detect the effects of stress was carried out and the sampling was performed at:
 - T0 = 0 h;
 - T1 = 2 h;
 - T2 = 8 h;
 - T3 = 24 h;
 - T4 = 48 h;
 - T5 = 72 h.

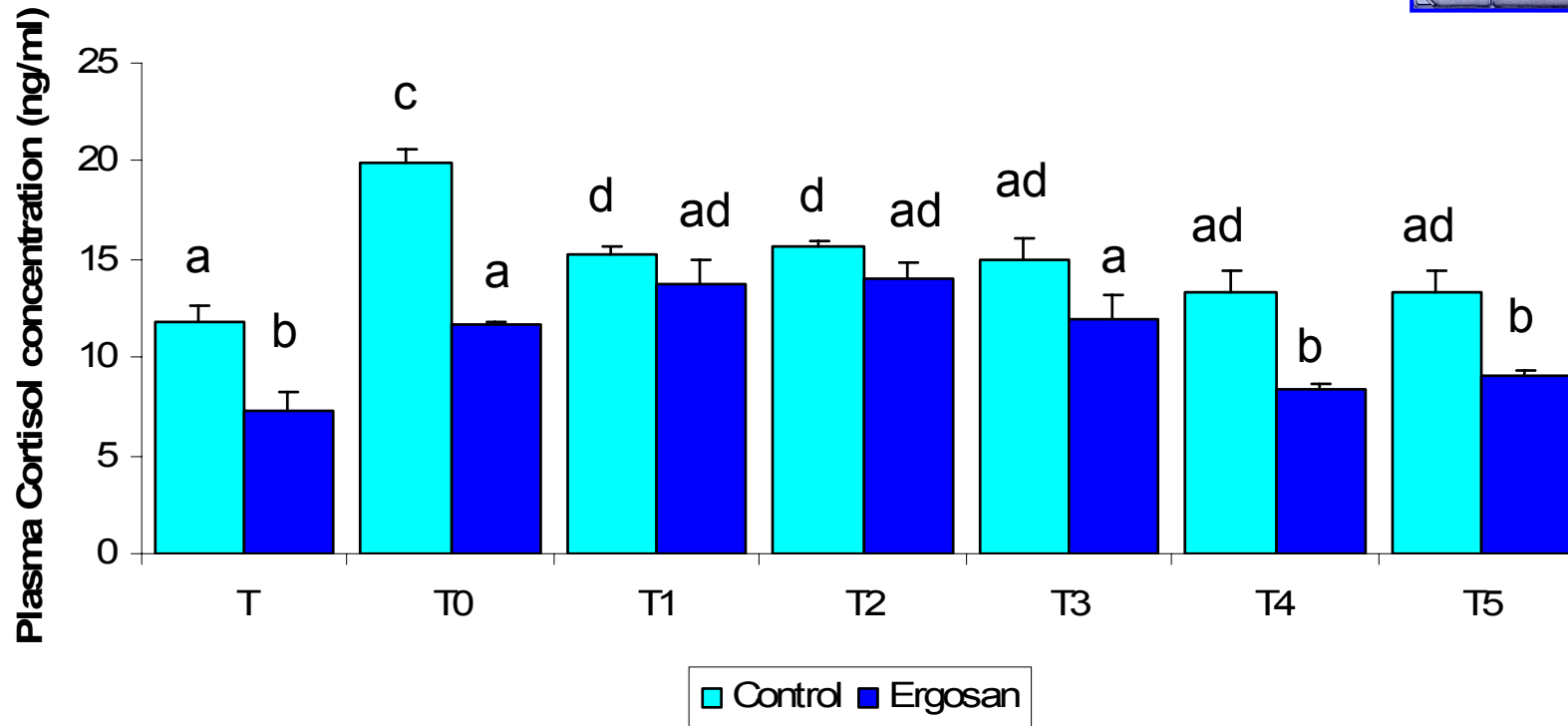
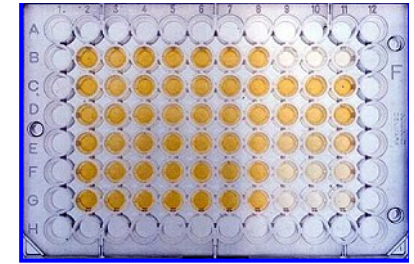
**EFFECTS OF ERGOSAN ON PHYSIOLOGICAL
AND CELLULAR RESPONSE AFTER HEAT
SHOCK STRESS**

Cellular response:



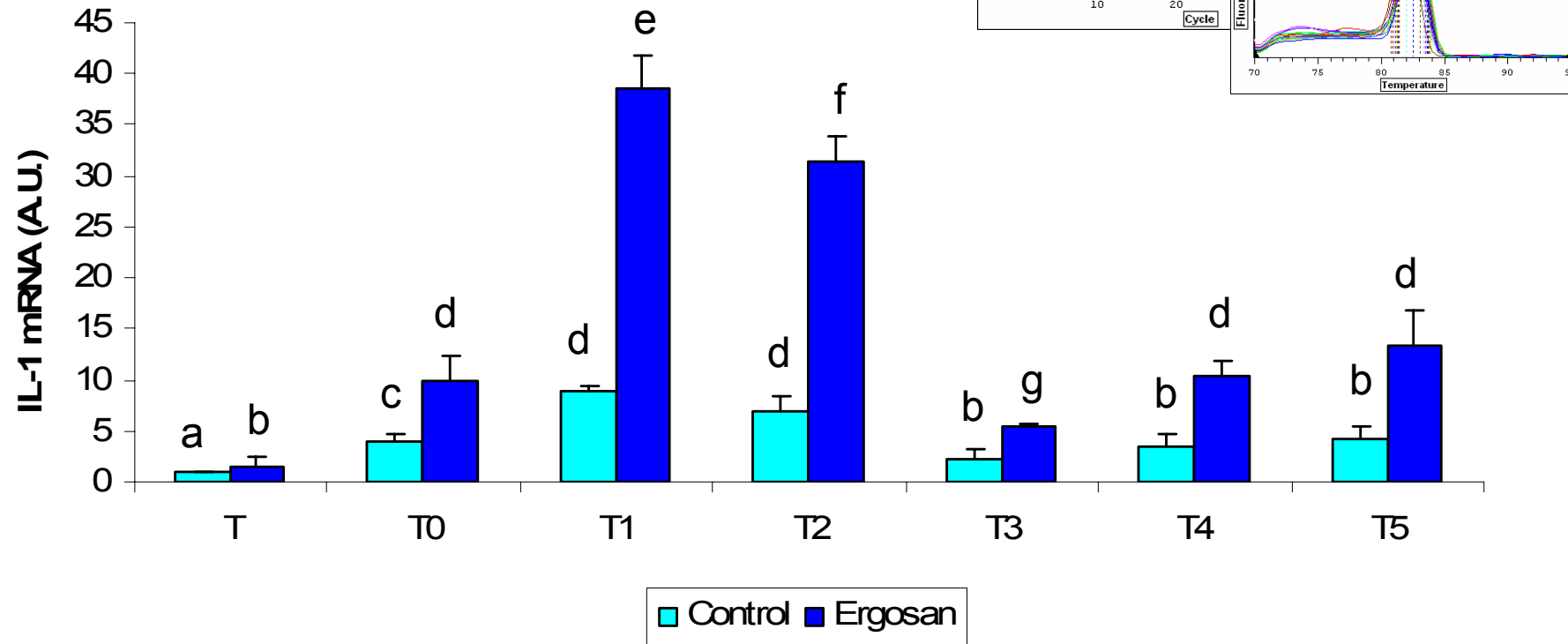
The levels of HSP70 mRNA in fish under heat shock stress significantly increase during the first 8 h. Lower levels of Hsp70 gene were found in controls fish respect to those fed by Ergosan from T0 to T2 (within the first 8 h), after 24h the treated groups showed lower Hsp70 levels respect to the control

Physiological response:



The cortisol levels in fish under heat shock stress reach its maximum at T0 in both groups, the fish fully recovered after 48 hours post stress, the lower levels of cortisol found in fish fed by Ergosan respect to the control were significant at T0, T4 and T5

Innate immunity: IL-1

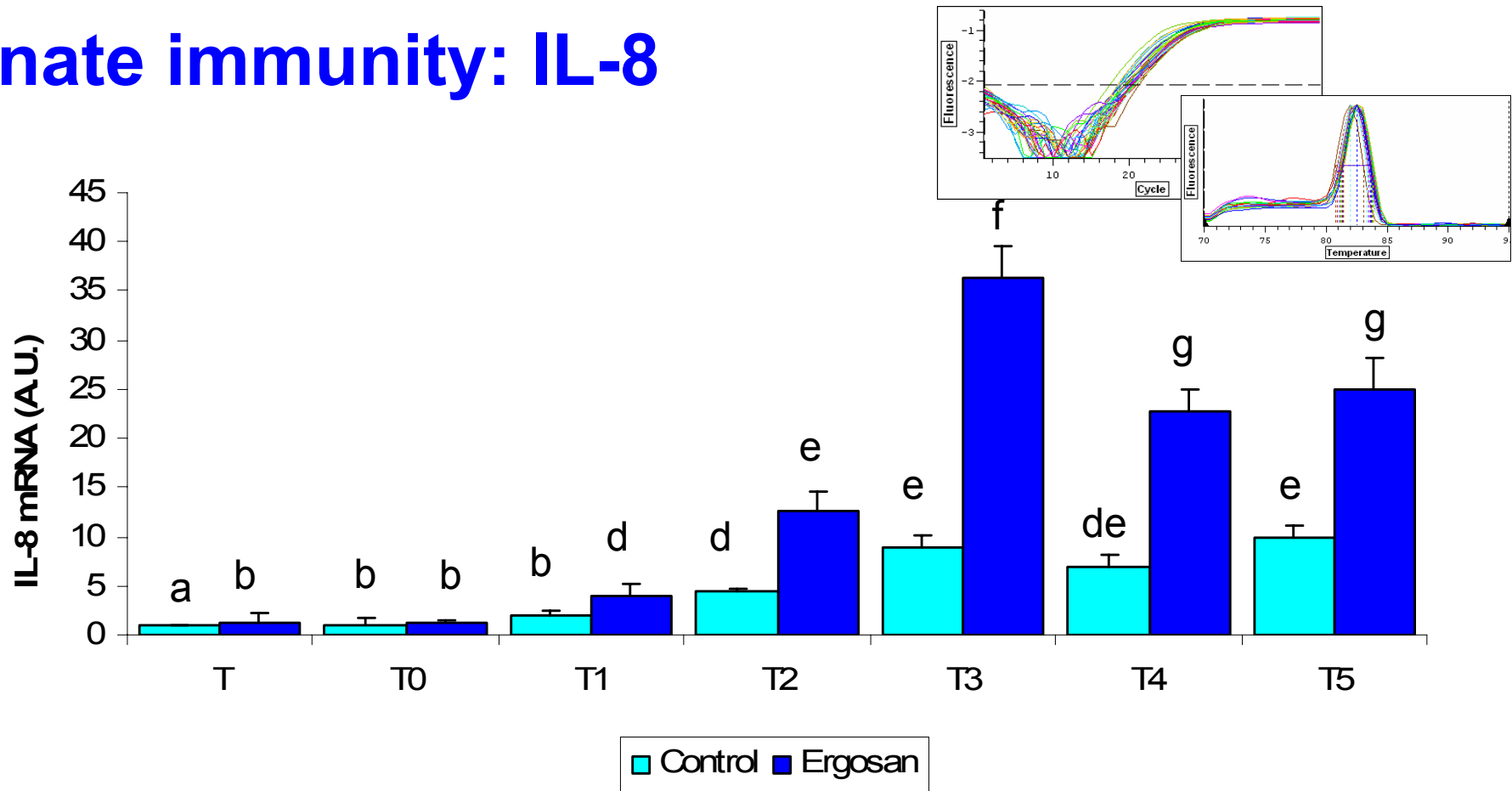


The IL-1 gene expression was significantly higher during all the time course in fish feed with Ergosan.

The maximal response occurred within the first two hours, a sort of “down regulation” was found 24 hours post stress.

After 48 hours the immune system restarts producing the mRNA of this cytokine

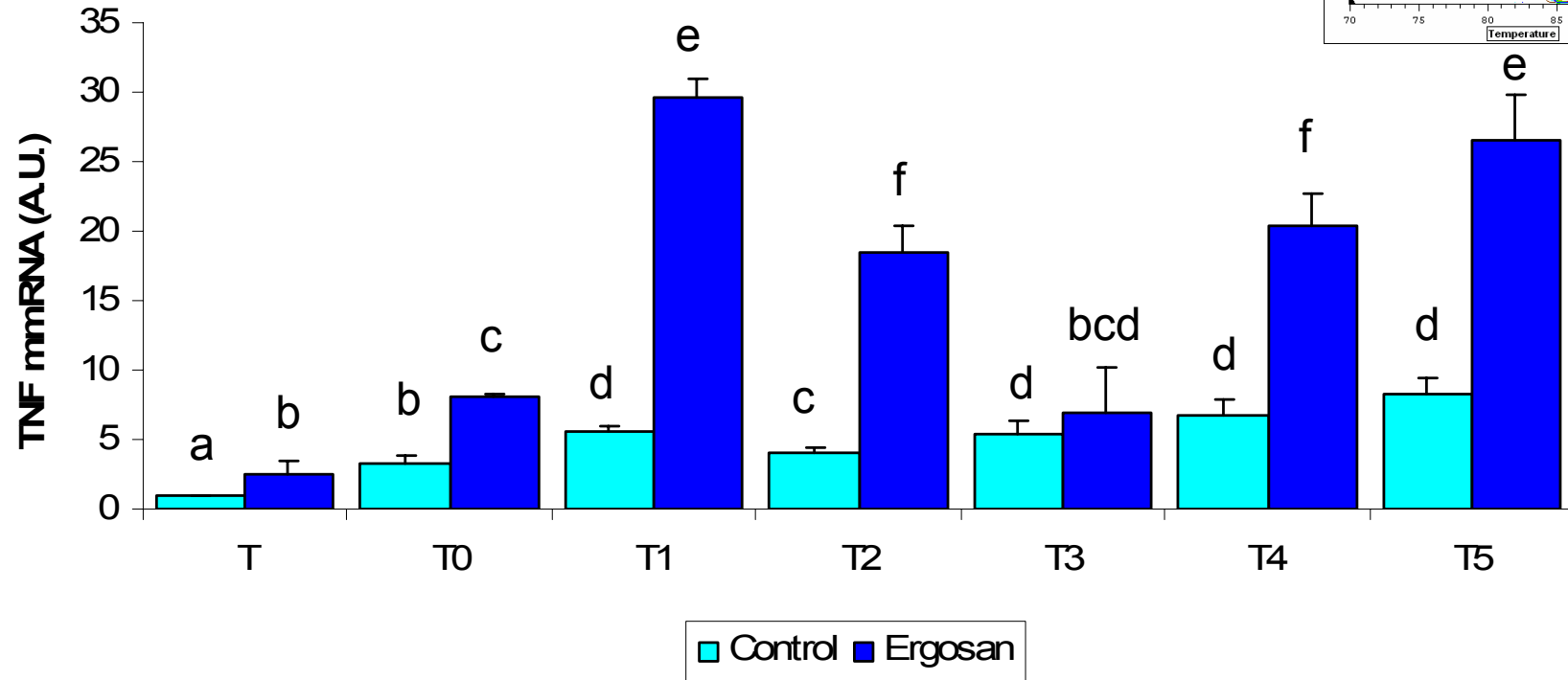
Innate immunity: IL-8



The IL-8 gene expression was significantly higher during all the time course in fish fed on Ergosan

IL 8 gene expression evidenced a maximum expression 24 hours post stress, the expression decreased during the following days

Innate immunity: TNF α



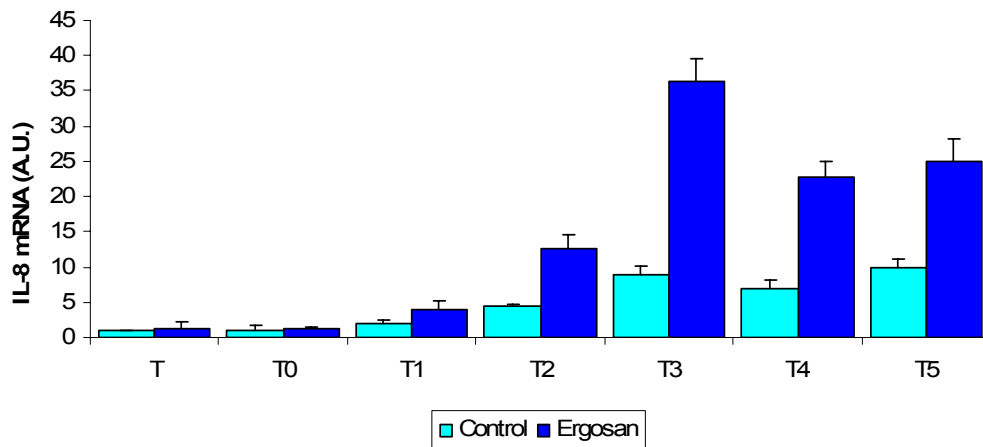
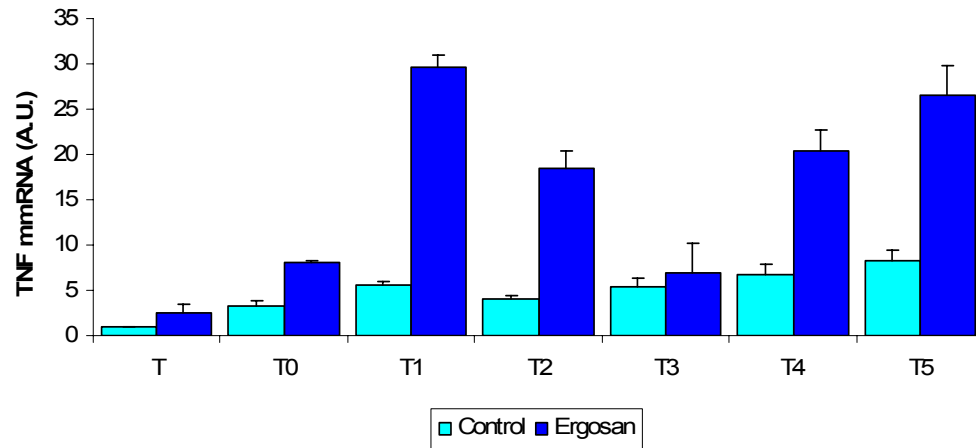
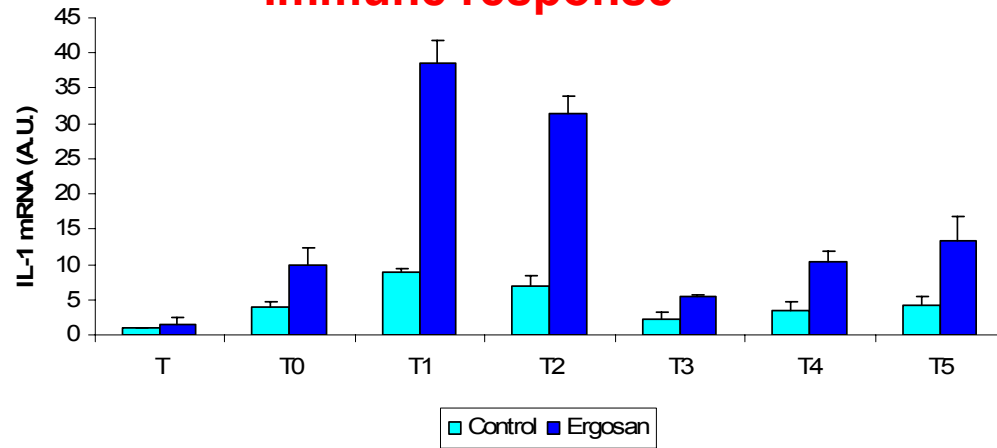
The TNF α gene expression was significantly higher during all the time course in fish feed with Ergosan.

The maximal response occurred within the first 8 hours, a sort of “down regulation” was found 24 hours post stress. After 48 hours the immune system restarts producing this cytokine

The higher levels of IL1, IL8 and TNF α found in all groups fed by Ergosan post heat shock stress, clearly evidenced the positive role of Ergosan on immunosystem modulation.

The results obtained confirm the induction of IL8 by IL1 and TNF α .

Immune response



HEAT SHOCK
STRESS

CONCLUSIONS

From our data, the positive role of Ergosan on fish welfare was clearly demonstrated:

- the role of **Ergosan** as immunomodulator is indicated by a more prompt immune replay. The expression of the 3 cytokines here examined was found significantly higher in fish fed on Ergosan than in control, showing a better adaptation to heat shock stress of this fish
- a better tolerance to rearing condition stress of **Ergosan** treated fish was evidenced

ACKNOWLEDGEMENTS

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Schering-Plough Animal Health Aquaculture. The Aquaculture Business Unit of the Schering-Plough Animal Health Corporation is a world leader in the discovery, development and marketing of pharmaceutical and biological products for the treatment and control of aquatic animal diseases. Through research and technical expertise, Schering-Plough Aquaculture enables fish farmers to improve production while helping ensure the sustainability of fish farming throughout the world'.

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