Effectiveness of a commercial Diatomaceous earth feed additive in mitigating transport stress in Sea Bass (*Dicentrarchus labrax).*

University of Bari - Aldo Moro
Department of Veterinary Medicine

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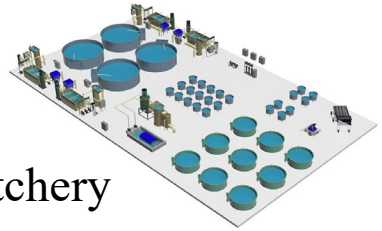
Incampo Giovanna, Calzaretti Giovanna, Centoducati Gerardo.

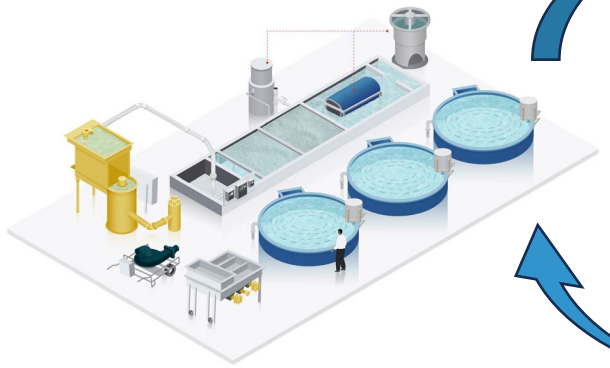
Fish transport



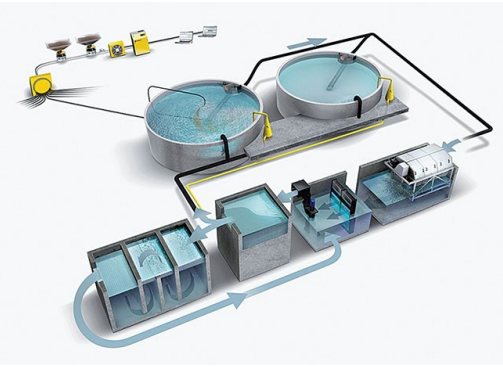
Hatchery

Fish farm

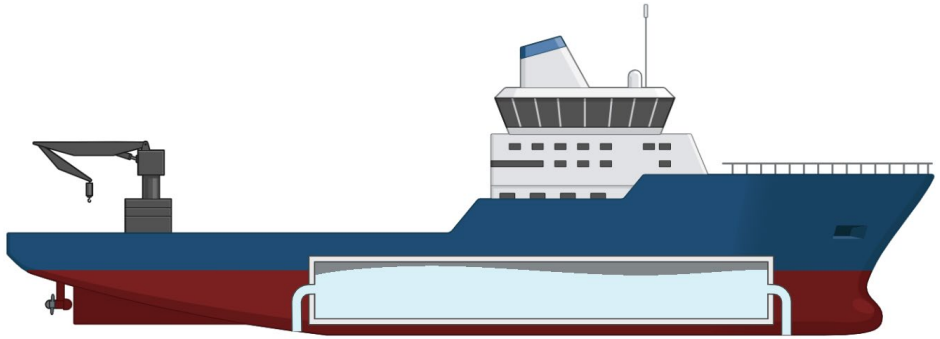
A common practice for the aquaculture industry.



Research centres



Modality of transport



Boat transport (Okada et al. 2015)

Short distance (Sampaio e Freire 2016)

Long distance (Hong et al. 2021)

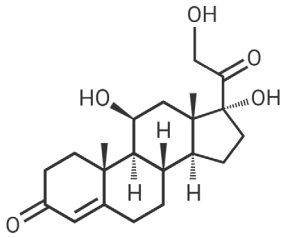
Air transport
(Vanderzwalmen M. et al. 2020)

Road transport (Manuet et al. 2014)

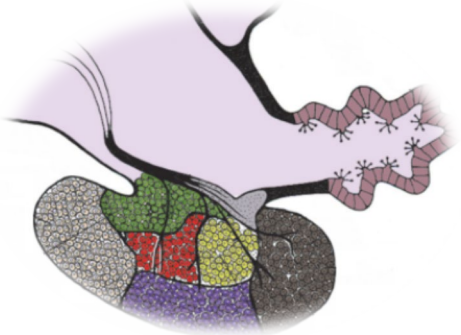
Stress axis

Hypothalamic pituitary interenal axis

(Schreck e Tort, 2016)

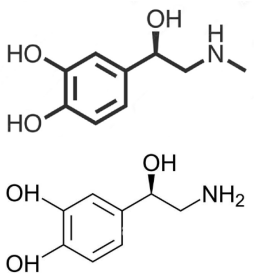


(Sopinka et al, 2016)



Hypothalamus sympathetic chromaffin axis

(Schreck e Tort, 2016)



(Sopinka et al, 2016)

Blood

- HCT (Zheng T., 2021)
- Hb (Zheng T., 2021)
- RBC (Zheng T., 2021)
- WBC (Zheng T., 2021)
- MCH (Shokr E.A.M., 2011)
- MCHC (Shokr E.A.M., 2011)
- Cortisol (Samaras et al., 2021)
- Lipidosis (Jerez-Cepa I., 2019)
- Hyperglycaemia (Jerez-Cepa I., 2019)
- Total Protein (Jerez-Cepa I., 2019)
- IgM (Guardiola. F.A., 2016)

Skin mucus

- Cortisol (Samaras et al., 2021)
- Glucose (Hussein. S.A., 2023)
- Lactate (Hussein. S.A., 2023)
- Total Protein (Zheng T., 2021)
- IgM (Guardiola. F.A., 2016)

Twofold Aim

To evaluate potential effect of a commercial product containing **diatomaceous earth (78%)** on stress, administered 10 days before transport to European sea bass (*Dicentrarchus labrax*) fry by assessing their effects on stress.

To assess potential stress indicators that are less invasive to evaluate, such as parameters related to skin mucus

VS.

Where?

Approved by the Ethical Committee for Animal Welfare of Animals University of Bari (approval no. 17/23)

Conducted in

Panittica Italia

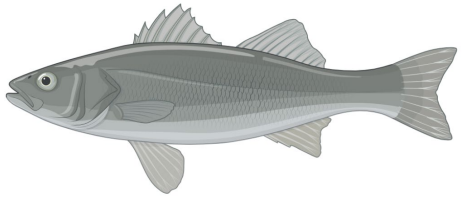
Società Agricola SRL

Road to Procaccio - 72016
Torre Canne di Fasano (BR)

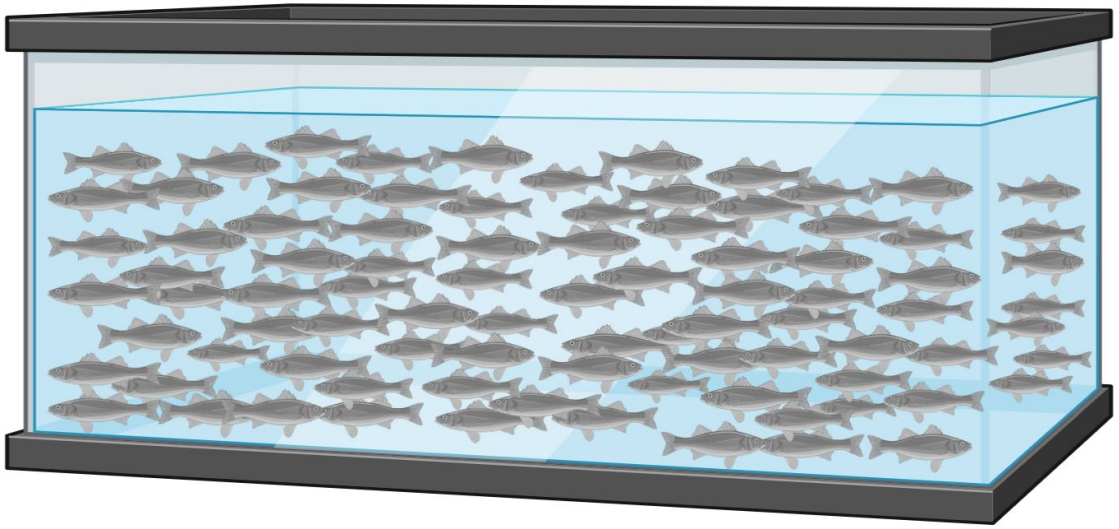
40°50'45.7"N 17°27'09.5"E

Experimental design

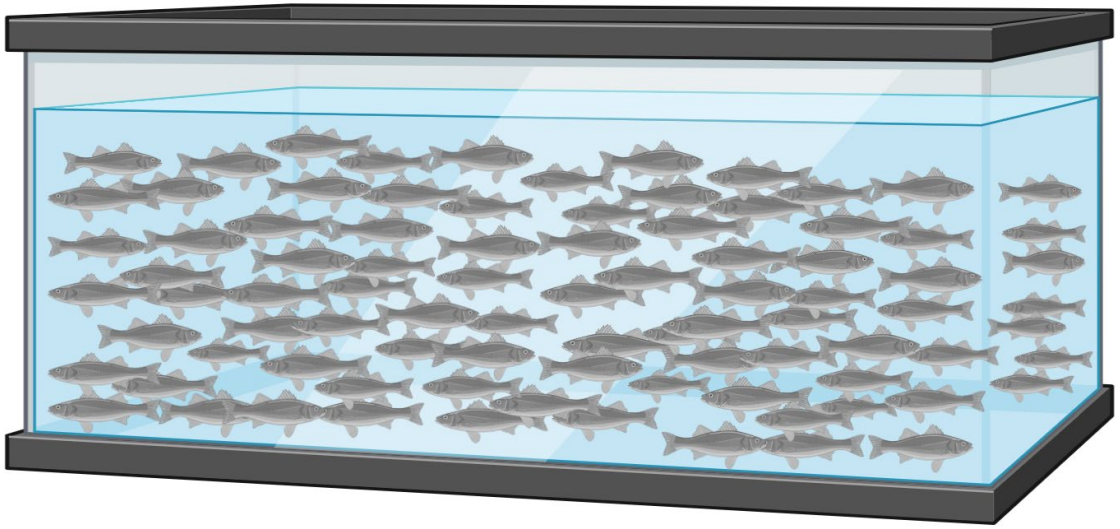
Sea Bass
(Dicentrarchus labrax)



- Average weight of 20.56 ± 1.30 g
- Average length of 13.06 ± 0.82 cm
- Tanks of 30 m^3
- Fish density of 35 kg/m^3
- 50.000 individuals



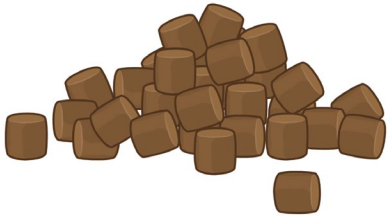
3 CONTROL TANKS



3 EXPERIMENTAL TANKS

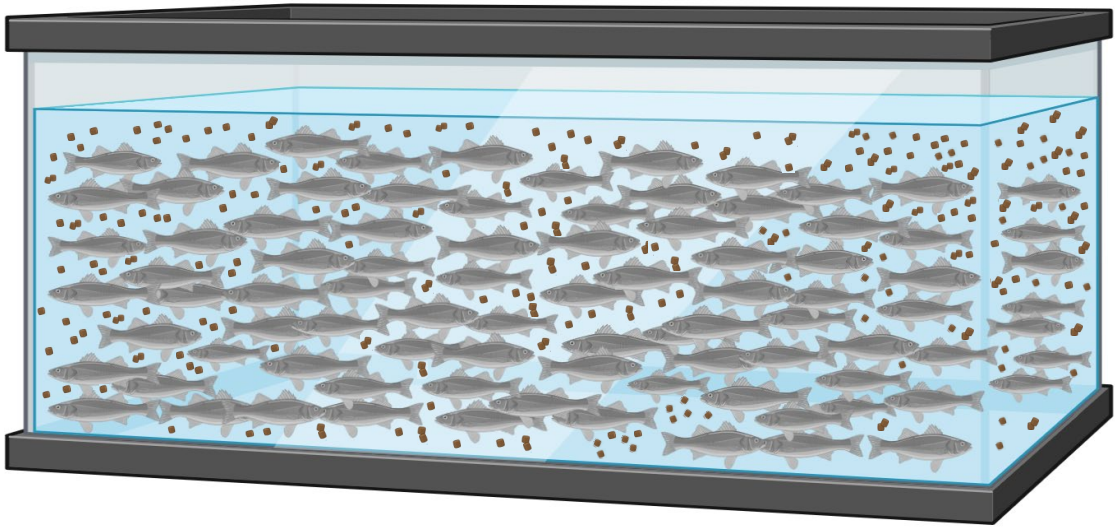
Acclimatization period

Commercial feed

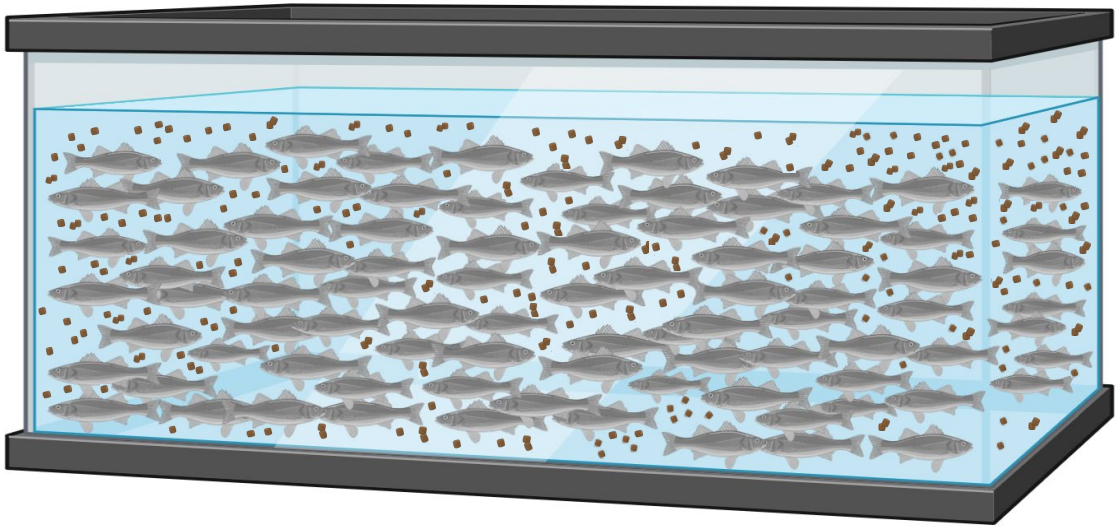


- SFR 3%
- 4 Time for day
- 20 days of Acclimatization

Commercial feed	
Crude protein (%)	51.0
Crude fat (%)	16.0
Crude fiber (%)	0.5
Total carbohydrates (%)	12.40
Ash (%)	10.0
Humidity (%)	8.4
Total phosphorous (%)	1.7
Dry matter (%)	91.6
Gross Energy (MJ/kg)	21.4
Digestible Energy (MJ/kg)	18.3



3 CONTROL TANKS

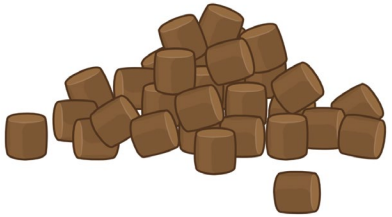


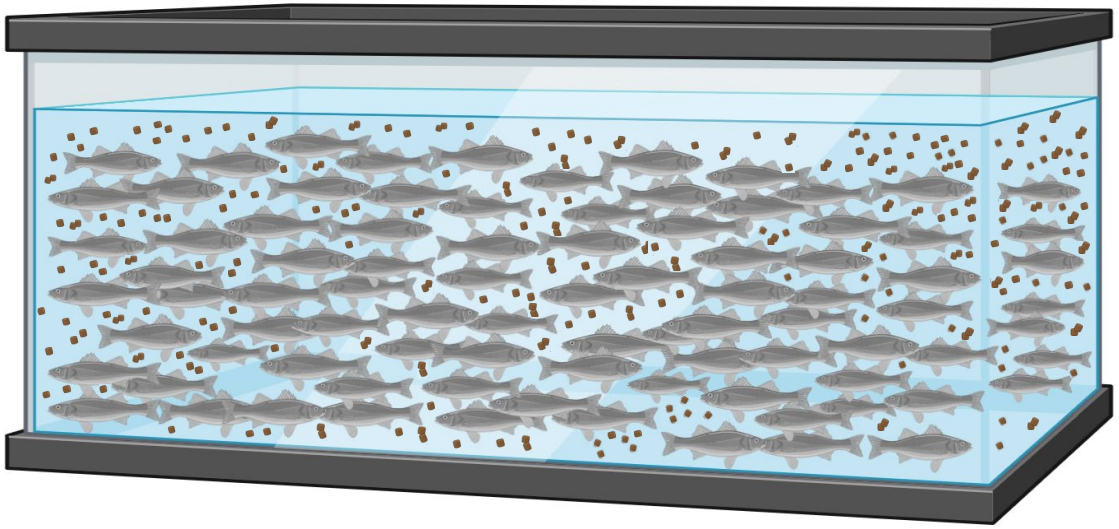
3 EXPERIMENTAL TANKS

Experimental design

- **10 days** before transport
- Feed supplemented with 0.8% (W/W)

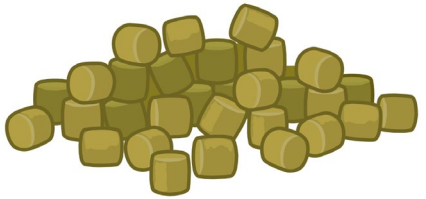
Commercial feed



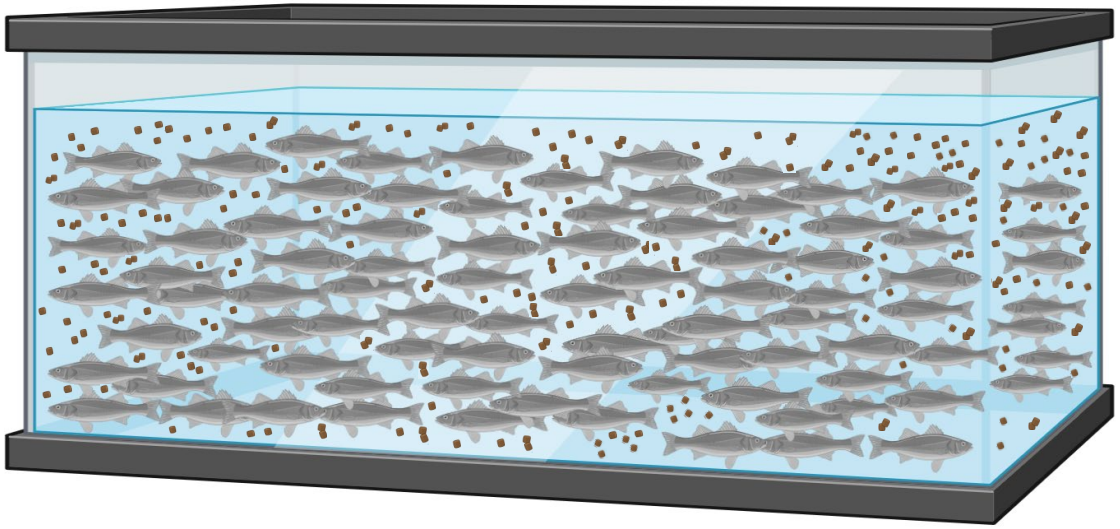


3 CONTROL TANKS

Experimental feed



- **Diatomaceous earth (78%)**
- Saccharomyces cerevisiae
- Silicic acid
- Polyphenols

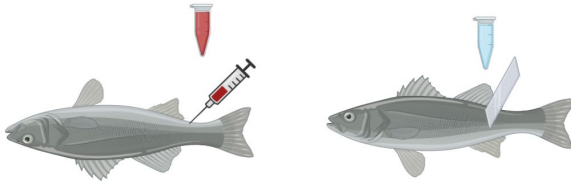


3 EXPERIMENTAL TANKS

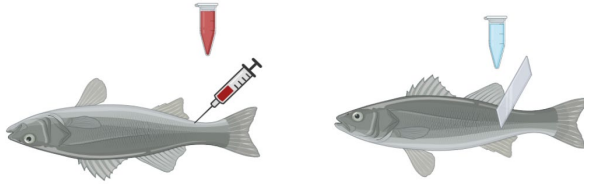
Simulation transport

- Fish were fasted for 20 h
- 6 Tanks (3 m^3)

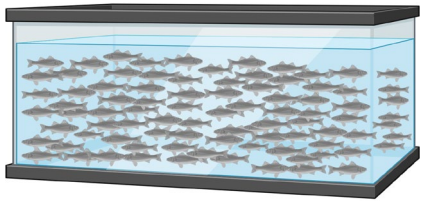
Blood *Skin mucus*



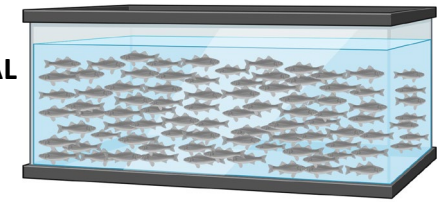
Blood *Skin mucus*

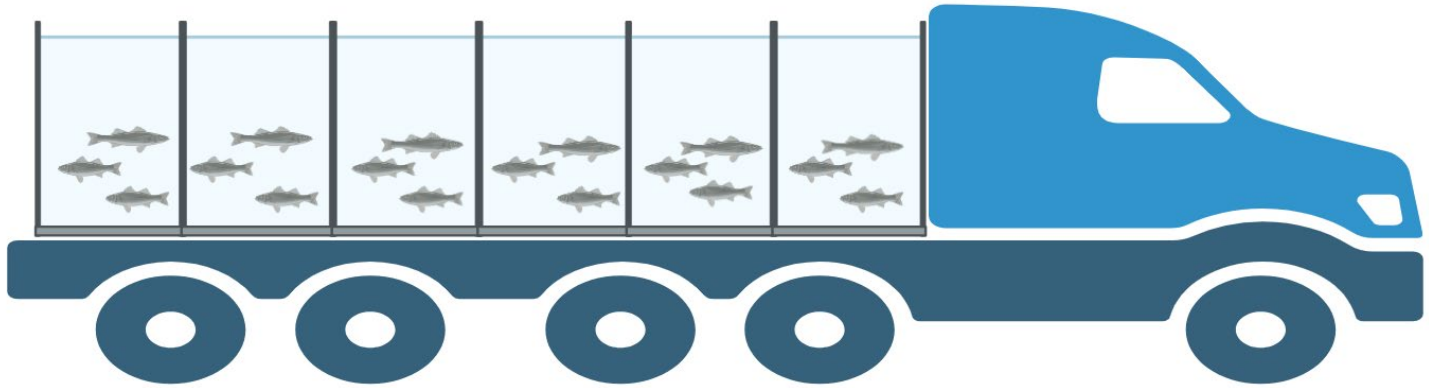


CONTROL TANK



EXPERIMENTAL TANK

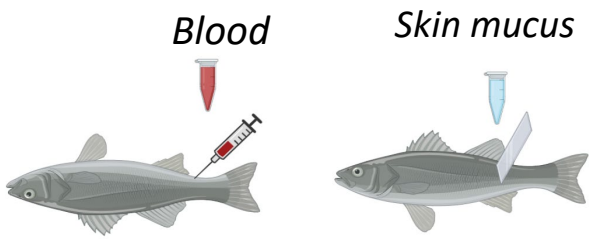




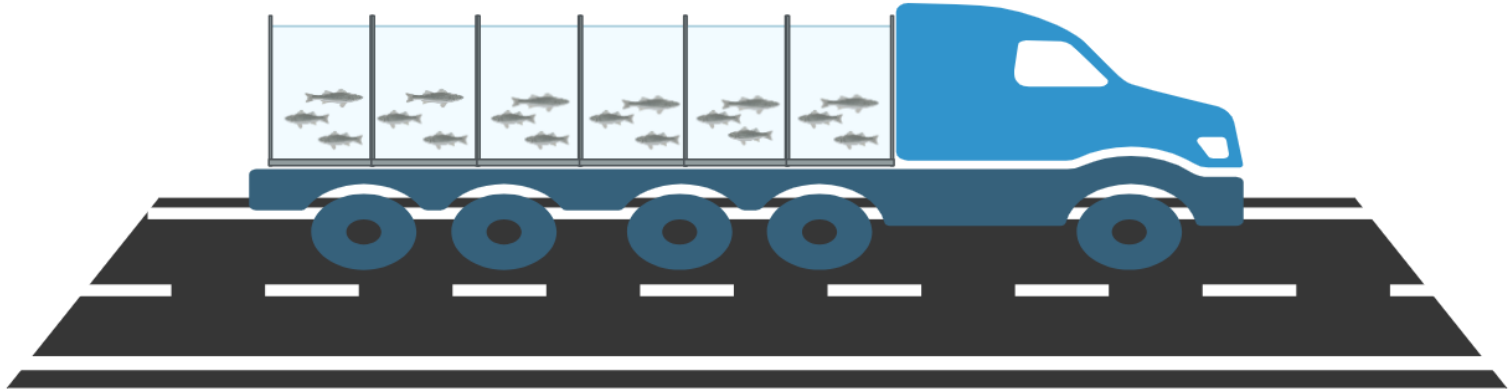
T0 = Basal values

T1 = Pre-transport values

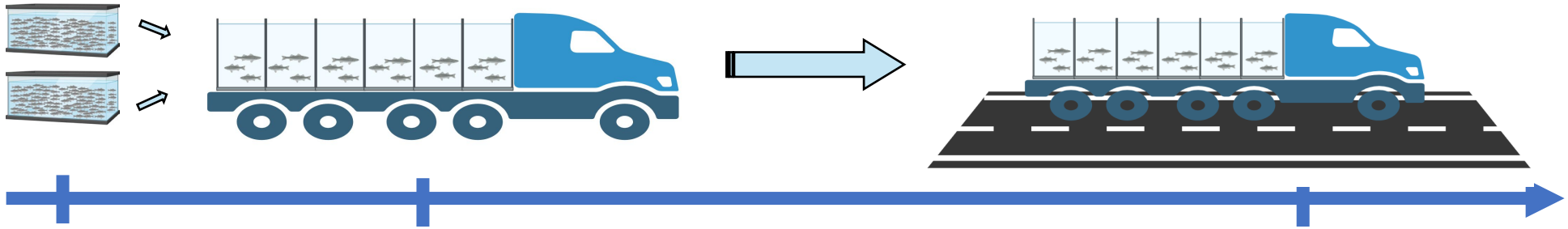
Simulation transport



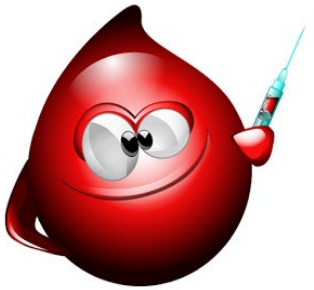
3 h

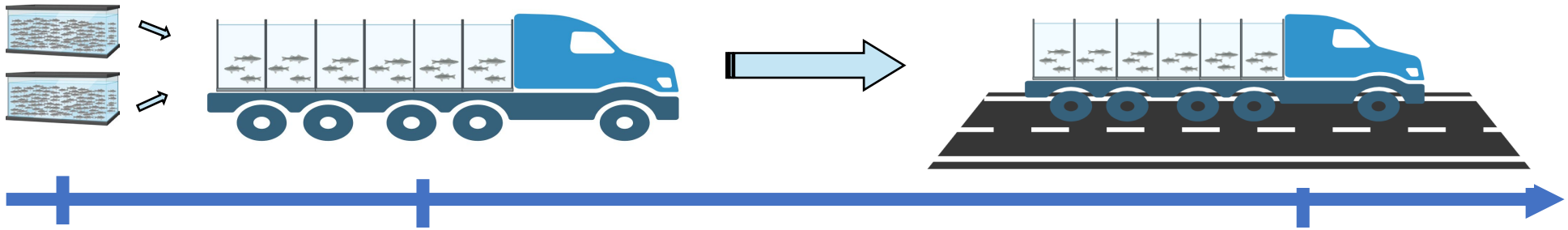


T2 = Post-transport values



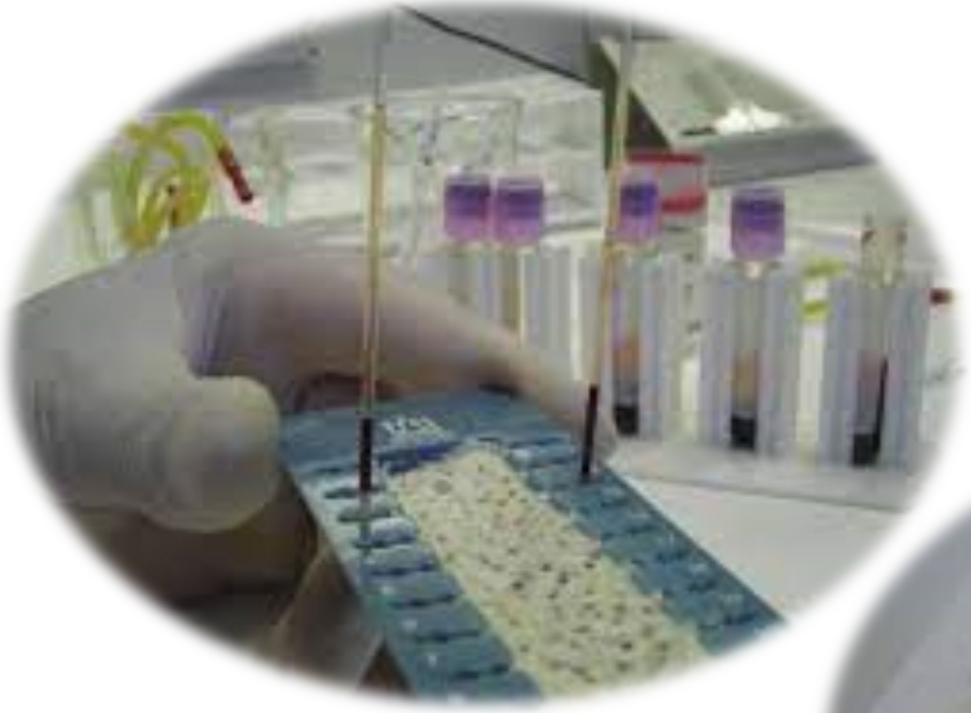
Blood collection





Skin mucus collection

Haematocrit determination

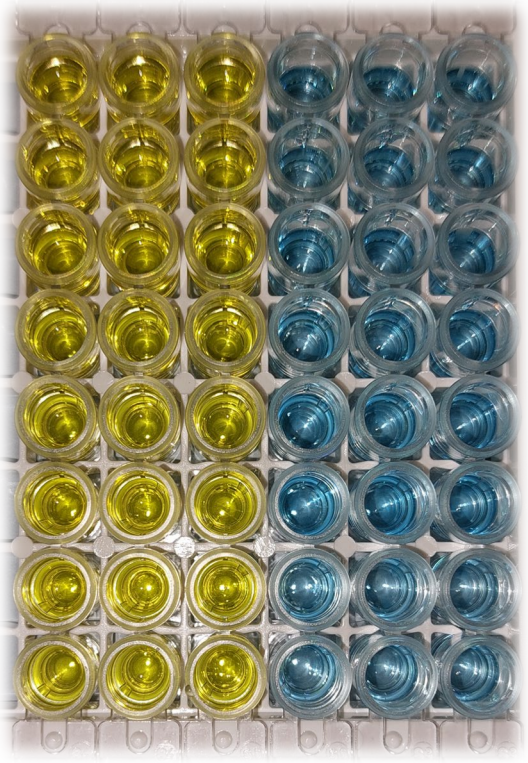


Immediately…!!!

Cortisol determination

Kit ELISA (DRG® Cortisol ELISA, DRG® International Inc, Germany)

- **Sample** Sierum/Plasma
- **# di wells** 96 wells
- **Range** 1.3 – 800 ng/mL
- **Sensitivity** 2.5 ng/mL (6.0 nmo/L)
- **Sample volume** 20 uL



Samaras et al (2017).
Samaras et al (2018).
Samaras et al (2021).

Absorbance was determined at 450 ± 10 nm with a microtiter plate reader.

Lactate, glucose, cholesterol, and triglycerides determination

Colorimetric kits (SPINREACT®, Girona, Spain)

Samaras et al. (2016);
Fernández-Alacid et al. (2018)

Statistical analysis

$$y_{ij} = \mu + \alpha_i + G_J + T_K + (G \times T)_{jk} + \varepsilon_{ijk}$$

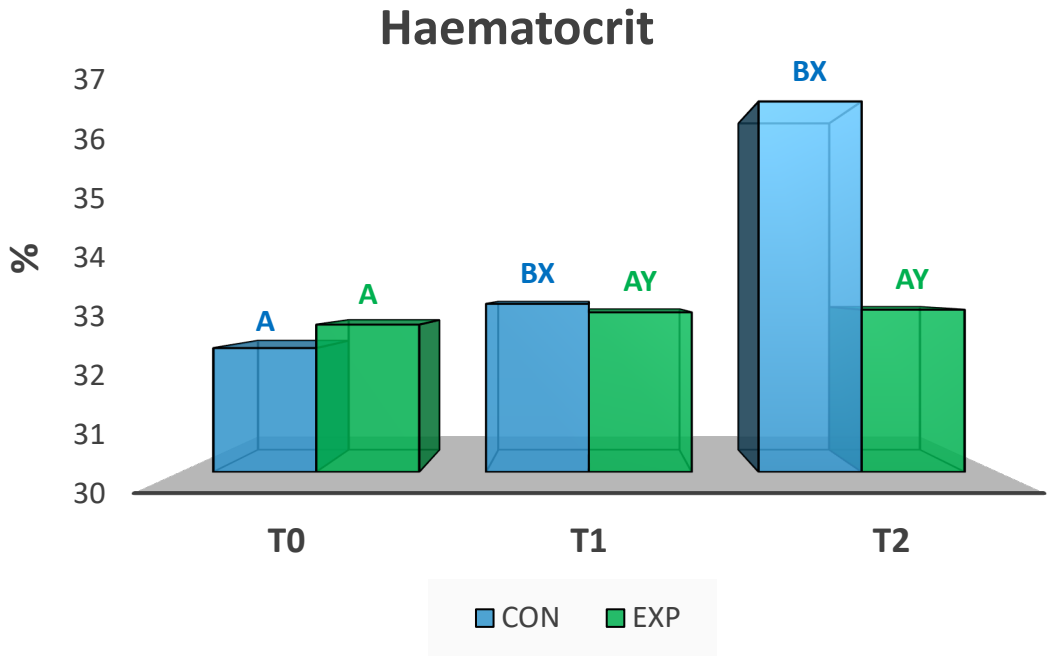
Analysis of Variance : ANOVA

***p* < 0.05**

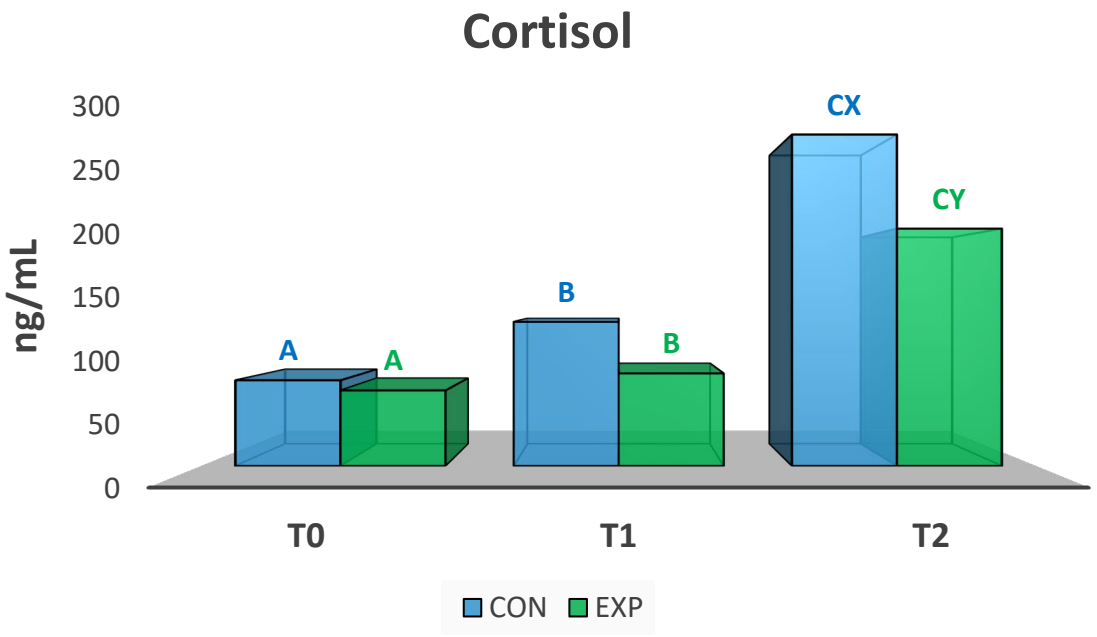
y_{ij} = parameters
μ = mean
α_i = random effect (i = 30)
G_j = group effect (J=2)
T_k = time effect (k=3)
$(G \times T)_{jk}$ = Group * time
ε_{iJk} = error

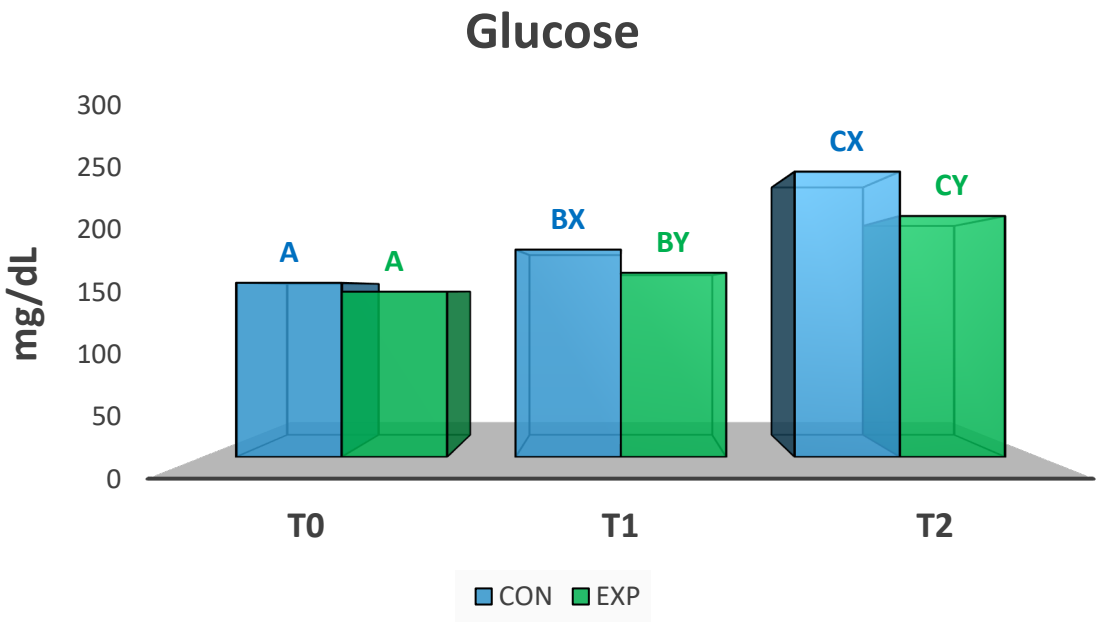
Pearson's correlations

Blood index

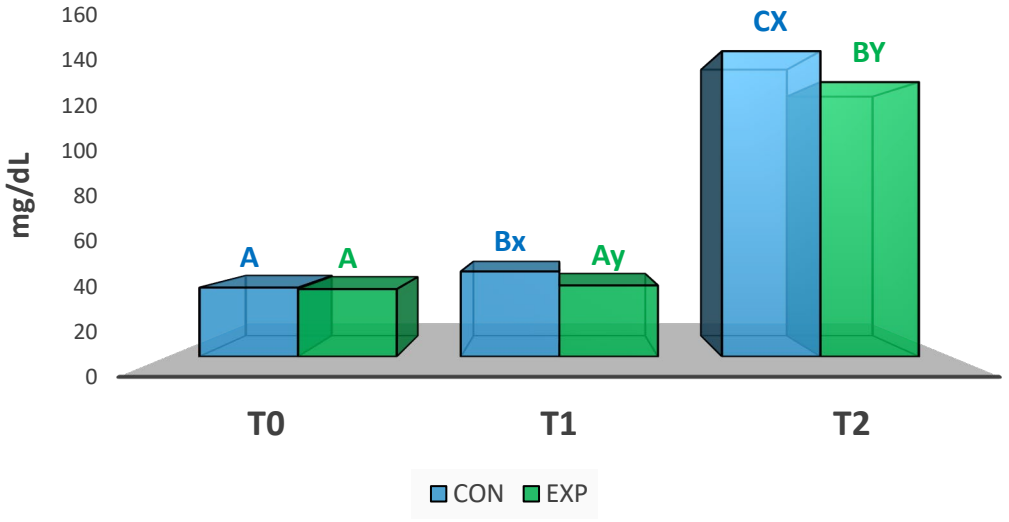


Different superscript letters with same colour (CON or EXP) show statistical differences (A - C: $P < 0.01$). Different superscript letters in the same time (T0 -T2), for each investigated pattern, show statistical differences (X, Y: $P < 0.01$; x, y: $P < 0.05$).

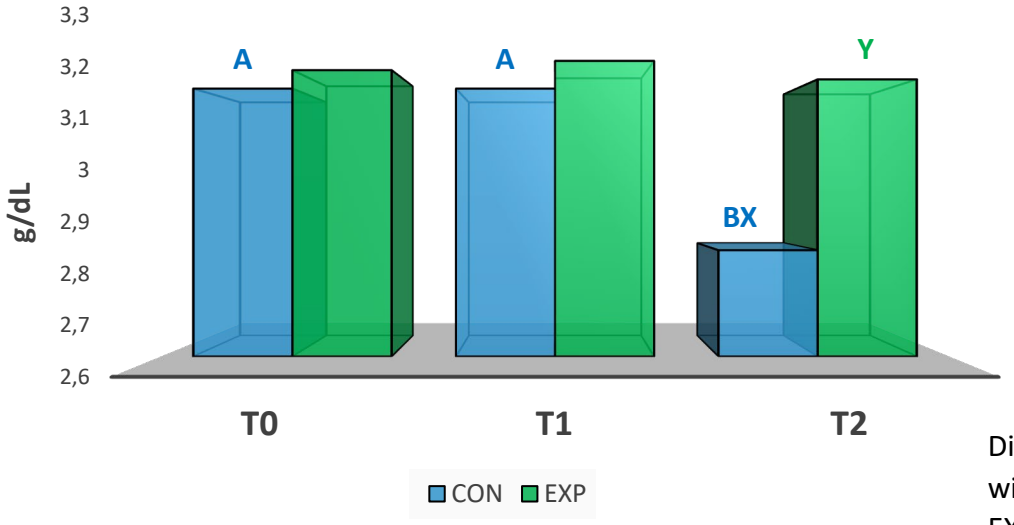




Lactate



Total Protein



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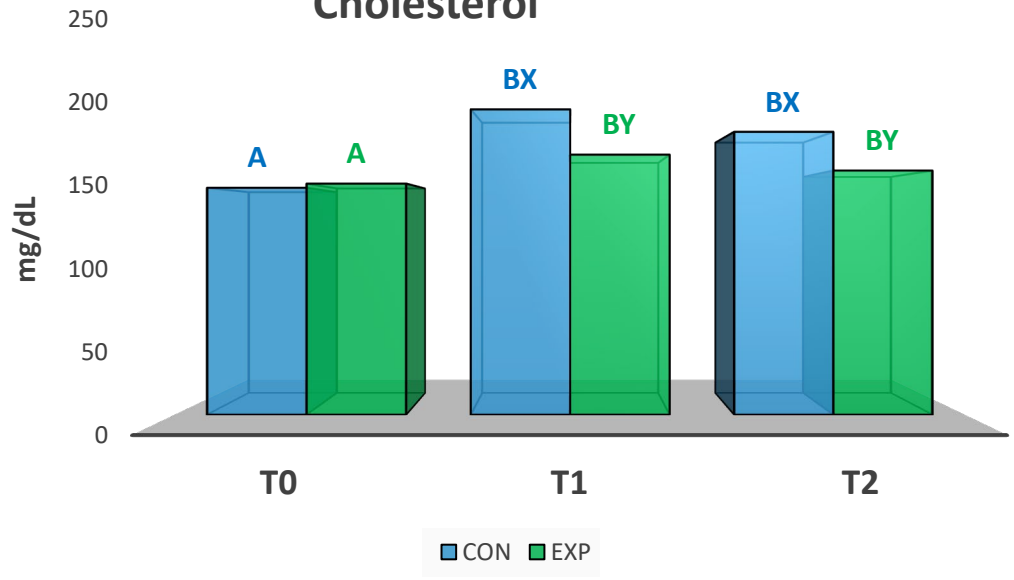
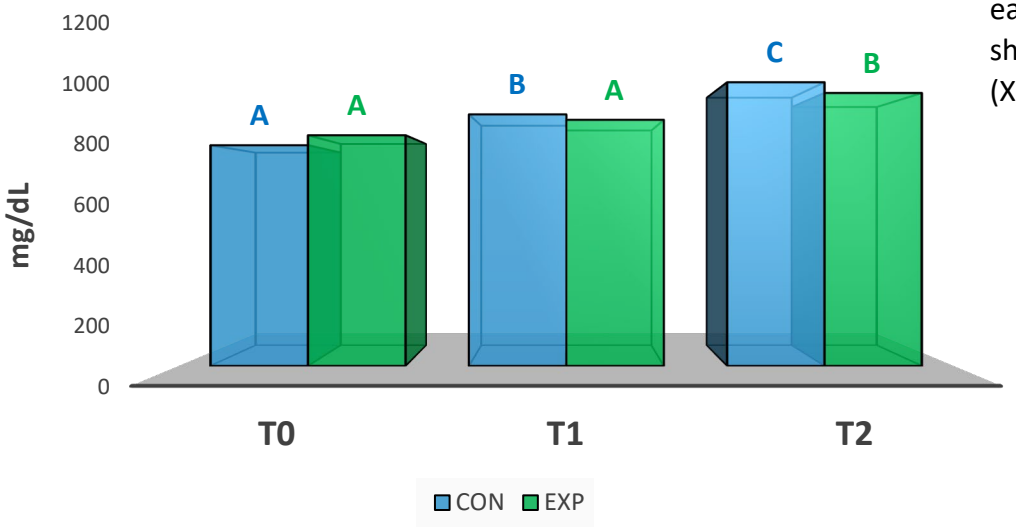
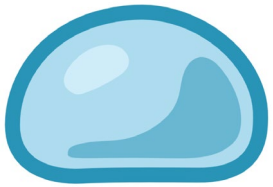
Cholesterol

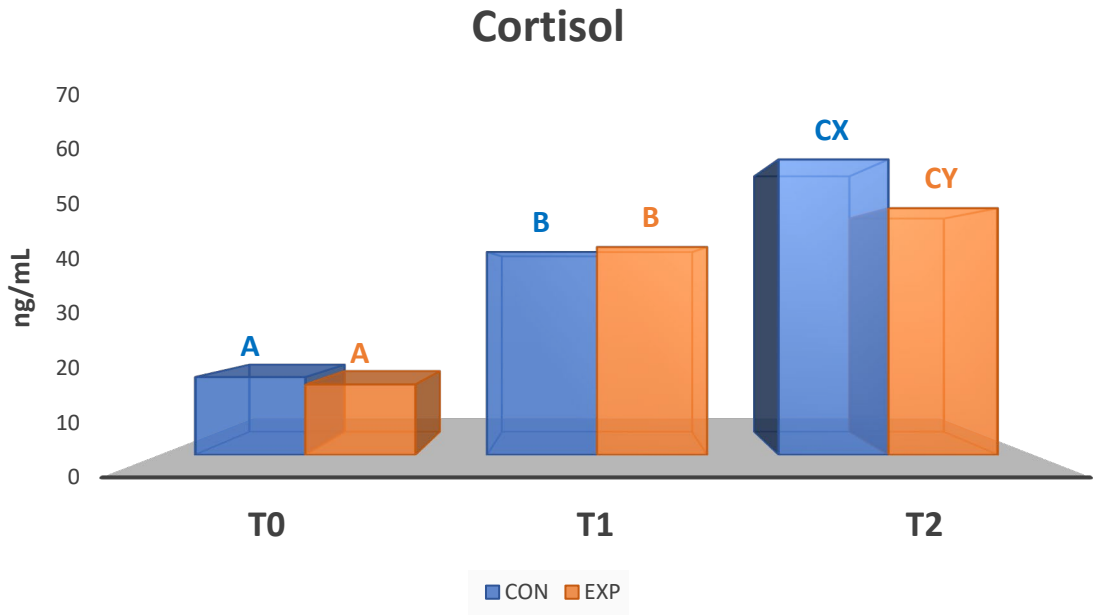


Triglycerides



Skin mucus index





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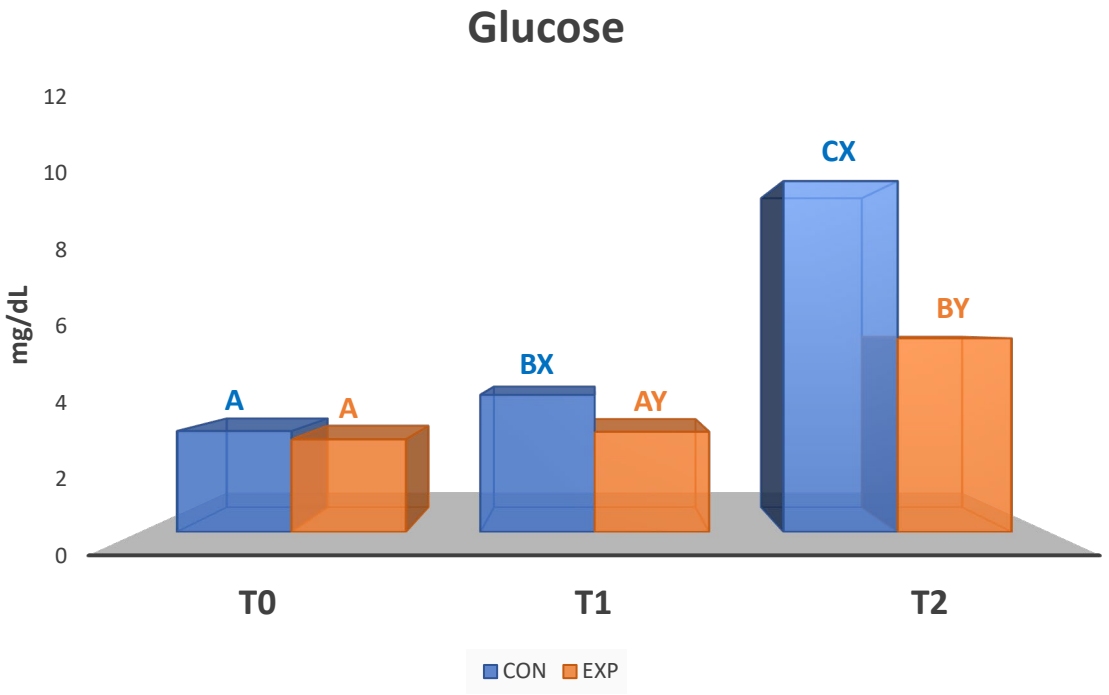
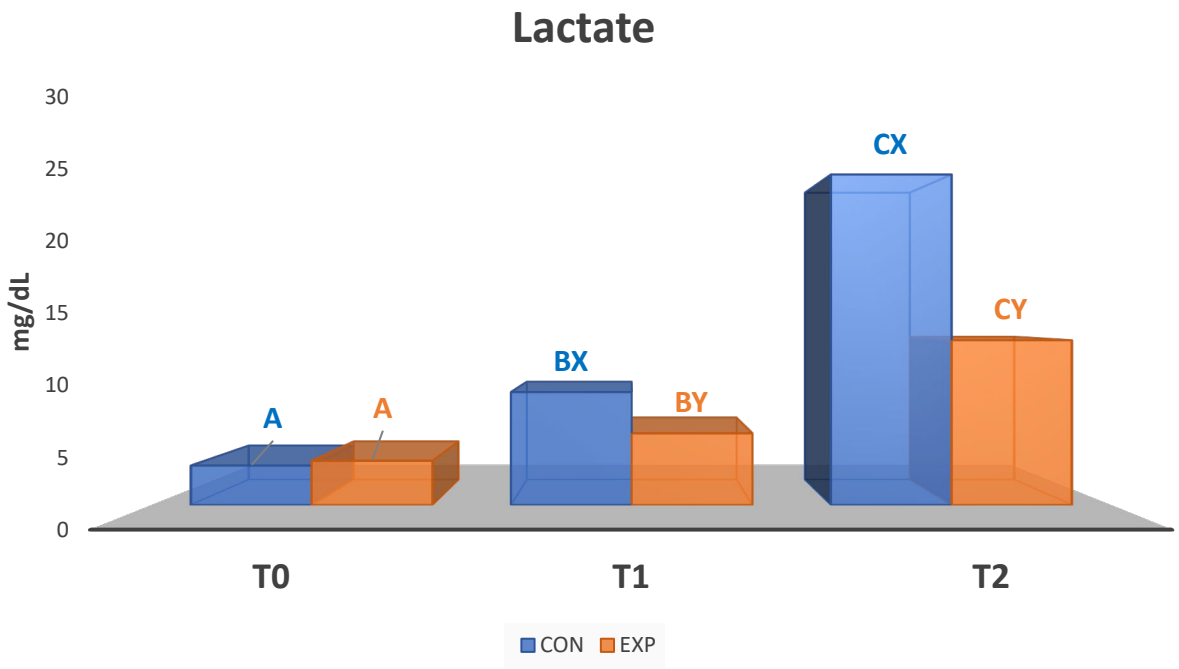
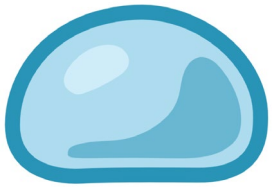
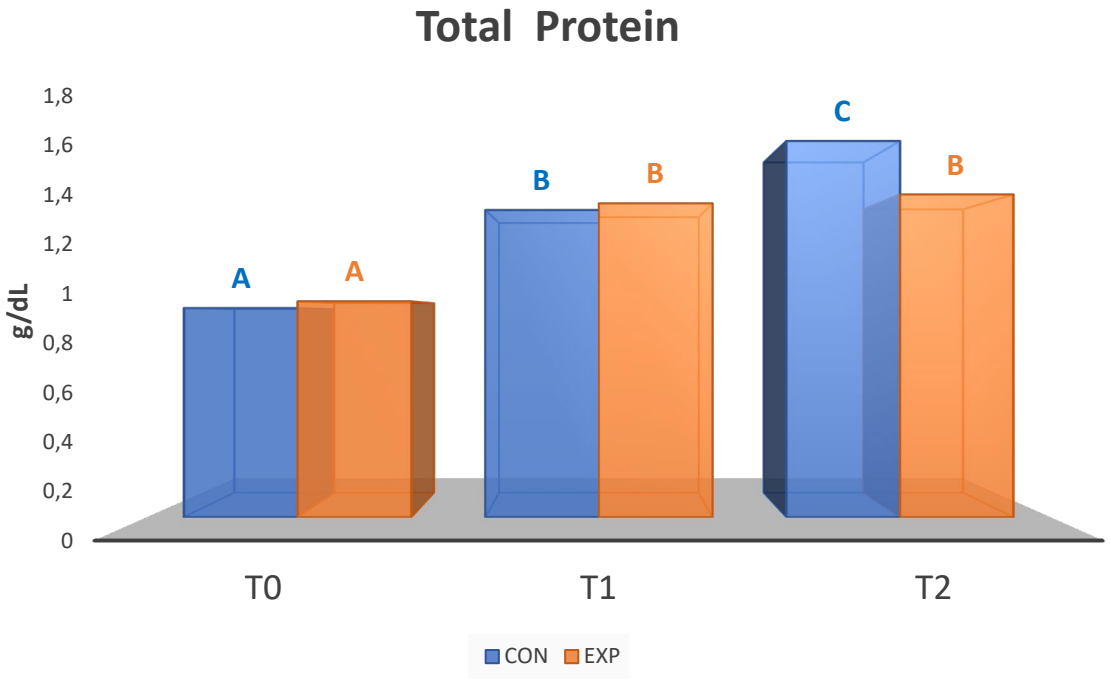




Skin mucus index





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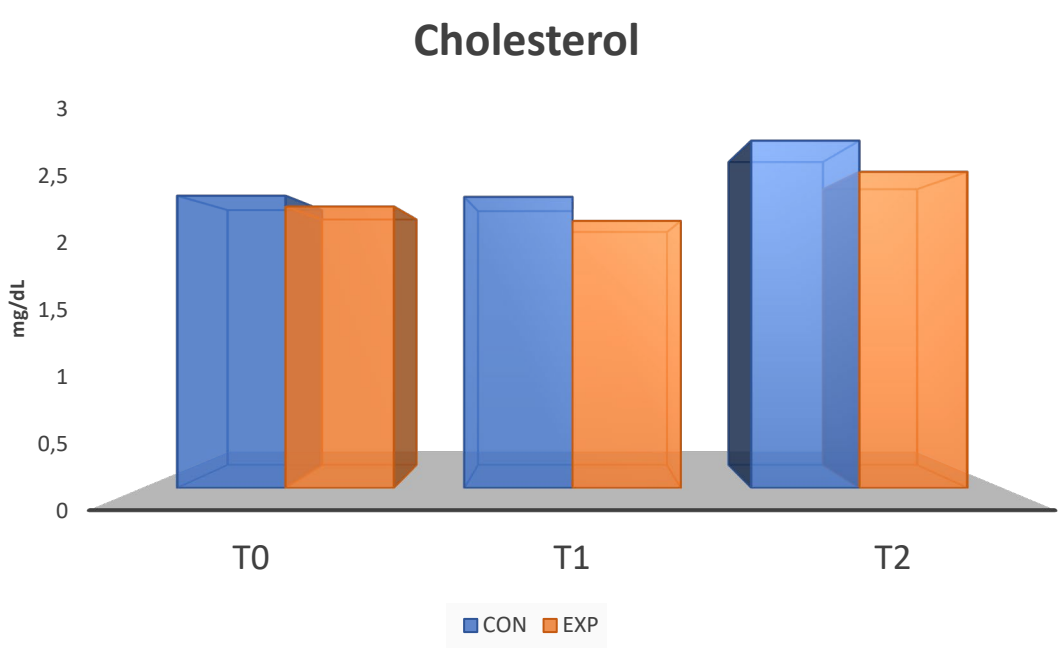
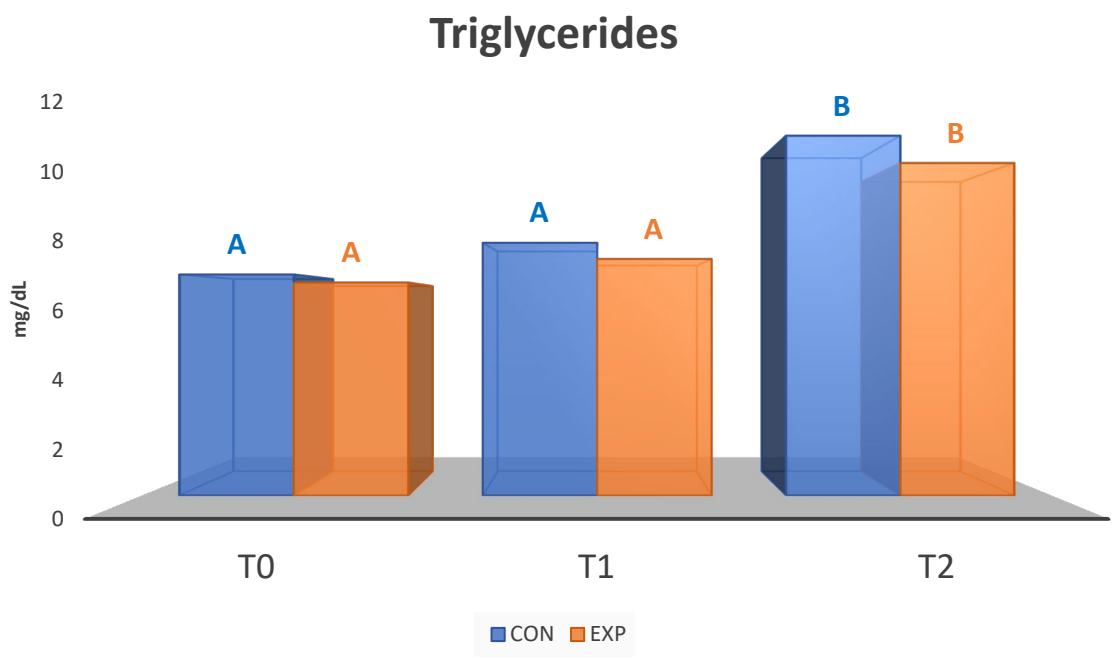
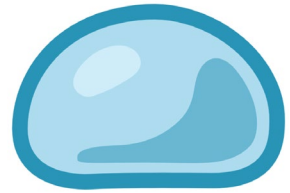


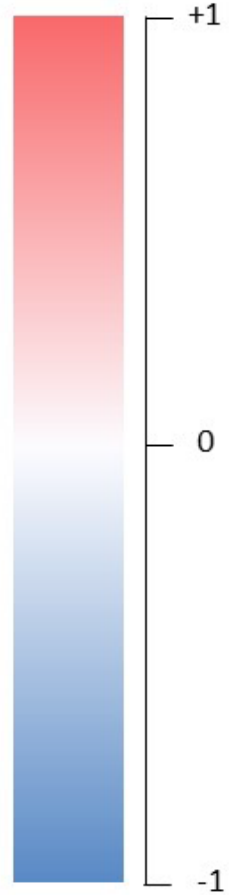


Pearson's correlation

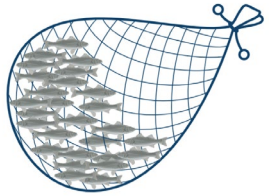


Skin mucus

Blood	Cortisol	Glucose	Lactate	Total protein	Triglycerides	Cholesterol
Cortisol	0,76798 (<.0001)	0,82893 (<.0001)	0,86837 (<.0001)	0,37334 (<.0001)	0,66449 (<.0001)	0,17174 (0.0755)
Glucose	0,77012 (<.0001)	0,86221 (<.0001)	0,80000 (<.0001)	0,45088 (<.0001)	0,63781 (0.0002)	0,19508 (0.0430)
Lactate	0,68591 (<.0001)	0,81857 (<.0001)	0,86927 (<.0001)	0,36020 (<.0001)	0,67815 (0.0035)	0,25363 (0.0081)
Total protein	-0,36539 (<.0001)	-0,46462 (<.0001)	-0,47449 (<.0001)	-0,13660 (0.15850)	-0,35202 (0,0002)	-0,27487 (0,0040)
Triglycerides	0,66104 (<.0001)	0,47831 (<.0001)	0,43520 (<.0001)	0,29725 (0.0001)	0,44612 (<.0001)	0,02578 (0,79110)
Cholesterol	0,58437 (<.0001)	0,33898 (0,0003)	0,40735 (<.0001)	0,36436 (0.0001)	0,22902 (0.01710)	0,02292 (0,8139)

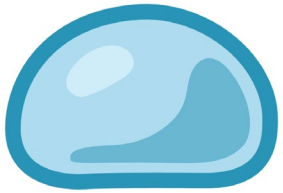


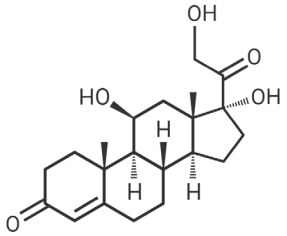
Conclusion

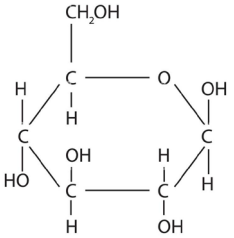


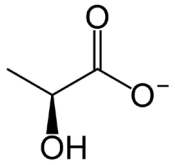
Handling and **transport** are stressful events for fish

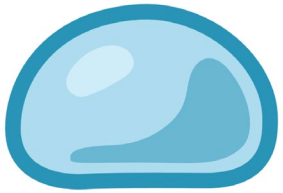
Skin mucus is matrix for measuring **cortisol, glucose** and **lactate** levels











Skin mucus is an ideal, **easy** and **less invasive** matrix for measuring cortisol, and secondary stress indices.

Diatomaceous earth has proven to be an excellent product for the mitigation of transport stress

Thank