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# Animal welfare in brown trout farming: hematological results

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**RIASSUNTO** – Benessere animale nell'allevamento della trota fario: risultati ematologici. *Questa sperimentazione riguarda i parametri ematologici come indicatori di benessere nell'allevamento della trota fario. Sono stati effettuati due cicli sperimentali, il primo da marzo a luglio 2004, il secondo da settembre a novembre 2004. Durante il primo periodo i livelli del trattamento sperimentale sono stati 3: densità di allevamento di trota fario 5, 10 e 20 kg/m$^3$. Sono stati rilevati cortisolo e glicemia. Durante il secondo periodo sperimentale son cambiate le densità di allevamento, corrispondenti a 10, 30 e 60 kg/m$^3$ (LD, MD e HD), sono stati rilevati emato crito, glicemia, colesterolo e cortisolo. I risultati ottenuti indicano che ci sono differenze significative nel caso del*

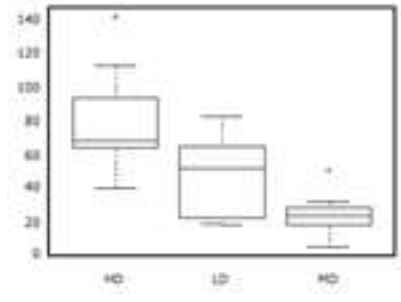
*l'ematocrito (%). Nel caso del cortisolo (ng/ml) si sono registrate differenze significative nei tre gruppi sperimentali tra i valori medi (LD = 48,4±25,1; MD = 24,2±11,9; HD = 79,5±30,7), tuttavia i livelli più bassi sono stati rilevati nel gruppo sperimentale a densità intermedia.*

**Key words:** fish welfare, brown trout farming, haematology.

**INTRODUCTION** – The effect of stress resulting from fish farming has received considerable attention in this last period and fish welfare in aquaculture is a relevant topic, very important for the future of aquaculture (Watson *et al.*, 2004; Klinger *et al.*, 1996; Peres *et al.*, 2004; Ron *et al.*, 1995; Wagner *et al.*, 1995; Watson *et al.*, 1998). Brown trout farming is less developed then rainbow trout farming, but this kind of fish farming is increasing, mainly for fish conservation and restocking aquaculture.
The effect of fish density in fish farming is one of the most largely investigated topics because the direction for future intensive aquaculture is the increase of fish density. In recent years a progressive increase of fish density has been observed in every sector of fish culture, Salmon farming is clear example of this phenomenon.
The aim of this study is to evaluate the effect of fish density on some haematological parameters in brown trout farming.

**MATERIAL AND METHODS** – This research was conducted in a brown trout farm situated in Morgex (Aosta valley, NW Italy) in two periods, ranging from April 2004 to November 2004. The first experimental trial lasted from April 2004 to August 2004 and the second from September 2004 to November 2004.
The experimental plan was balanced monofactorial, the experimental factor was fish density stocking with three levels of treatment. In the first period the selected fish densities were: 5 kg/m$^3$, 10 kg/m$^3$ and 15 kg/m$^3$; in th second period the fish densities were 10 kg/m$^3$, 30 kg/m$^3$ and 60 kg/m$^3$. During all the experimentation dissolved oxygen and water temperature were measured, moreover the fishes were periodically weighted in order to maintain constant the initial fish densities during all the experimentation. The feeding ratio was 1.2% of biomass in the first period, in the second period it was initially 1% and it was progressively reduced to 0.5%.

In first trial the initial mean body weight was respectively g 234.8 (5 kg/m$^3$), g 206.6 (10 kg/m$^3$) and g 251 (15 kg/m$^3$). In second it was respectively g 517 (10 kg/m$^3$), g 509 (30 kg/m$^3$) and g 534 (60 kg/m$^3$). At the end of both



experimental trials, blood samples were taken and glucose, colestherol, ematocrit and cortisol were measured in 10 fishes per experimental treatment in the first trial and in13 fishes in the second trial.
Blood samples were taken from trout singularly anaesthetised with terbutilic acid (5 ppm/10 l) which is the 50% of normally used dose. The glucose level was immediately measured, cholesterol was analysed through an enzymatic test, ematocrit was analysed with microcapillar and cortisol with RIA kit DSL 2000. In May 2004 a little experimentation on acute stress was conducted in order to evaluate the effect of fish handling on haematological analysis before to start main experimentation.
We utilised 16 trout (body weight from 150 to 400 g), in 7 fishes we utilised the normal anaesthetic and in 9 fishes we induced strong acute stress by putting them out from the water for 4 minutes and taking the blood without anaesthetic.
We measured the cortisol in these fishes and we checked these fishes for 2 weeks after blood sampling in order to see if there was any difference in mortality rates.
For the statistical analysis of data, Shapiro Wilks normality test and Bartlett test homogeneity of variance were used in order to verify the correct application of ANOVA. When the basic assumption were respected, ANOVA was utilised, otherwise Kruskal Wallis test was used. Pearson correlation coefficient was utilised in order to investigate multiple correlations between haematological parameters.

**RESULTS AND CONCLUSION** – During the first experimental trial we observed some fish mortality not related with experimental conditions, consequently we made the second experimental period in autumn with an increased effect of stocking density.
Acute stress experimentation in May 2004 did not show fish mortality related to fish handling.
In the second period (September 2004 – November 2004) we didn't observe fish mortality. Only glucose and cortisol respected the normality of distribution of data ($p< 0.05$), moreover it is possible to observe that only for cortisol the homogeneity of variance was respected (Shapiro Wilk W=0.9225, P=0.02; Bartlett test K=8.1761, df=2, P=0.0167).
These preliminary tests clearly indicate that ANOVA can be correctly used only for cortisol data. As a far as cholesterol is concerned (LD: 208.8±54.9; MD: 252.1±61.9; HD: 237.3±63.4), the average difference in experimental groups is not statistically significant probably for high data variability. Ematocrit values generally indicate that there were not acute stress conditions during the blood sampling (LD: 26.8±6.2; MD: 32.6±5.6; HD: 30.9±4.6), in normal conditions ematocrit should be between 20 and 40% in fishes, moreover there is a statistically significant decrease for ematocrit in low density group ($p< 0.05$).
Glucose level values are not different in the experimental group (LD: 78.7±29.2; MD: 80.6±29.3; HD: 82.5±29.5), these values are similar to those measured in the previous trial (April 2004 – July 2004), there is no statistical difference between groups.
Cortisol values are different in the experimental groups (LD = 48.4 ± 25.1; MD = 24.2 ±11.9; HD = 79.5± 30.7) ($p< 0.05$), in particular it is possible to observe that the lowest level is in the medium density group (30 kg/m3) (Figure 1). Cortisol proves to be a more indicative haematological parameter for different experimental conditions.

Figure 1. Cortisol level (ng/ml) in the second experimental trial, according to the different stocking density:

HD high density (60 kg/m3);