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To cite this article: Paolo Melotti, Alessandra Roncarati, Lucia Angellotti1, Andrea Dees, Gian Enrico Magi, Claudio Mazzini, Carlo Bianchi & Rosanna Casciano (2004) Effects of rearing density on rainbow trout welfare, determined by plasmatic and tissue parameters, Italian Journal of Animal Science, 3:4, 393-400

To link to this article: http://dx.doi.org/10.4081/ijas.2004.393
Effects of rearing density on rainbow trout welfare, determined by plasmatic and tissue parameters

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Paper received June 7, 2004; accepted August 2, 2004

ABSTRACT

The work aimed to determine the welfare status of rainbow trout (50±15 g), intensively reared to commercial size in two concrete raceways (V1, V2) at different stocking densities, to reach final load of 40 kg/m³ and 20 kg/m³, respectively. Throughout the out-phase, plasma parameters and hepatic glycogen content were determined every three months and compared with those of rainbow trout of the same age and mean weight, reared extensively (VE). At the end of the trial significant differences of the monitored parameters were found between rainbow trout reared in raceways (V1, V2) and those held in the reservoir (VE). Plasma triglycerids, total cholesterol and alkaline phosphatase in fish reared intensively were significantly different from fish reared in extensive conditions. The highest levels of these parameters in V1 and V2 could be justified mostly by the administration of balanced feeding and consequent modifications of energetic metabolism and in small part to the high density of the intensive rearing conditions. Transaminases (AST, ALT) increased in V1 and V2 only at the first sampling. Afterwards, the activity of these two enzymes returned to the normal range at the subsequent assays, suggesting a capacity of the rainbow trout to adapt to the severe conditions of confinement, typical of out-basins. No significant differences among groups were observed for total protein, glucose, CK and LDH. A similar situation was also shown for liver glycogen content.

Key words: Rainbow trout, Welfare condition, Plasmatic parameters, Hepatic glycogen.

RIASSUNTO

EFFETTI DELLA DENSITÀ DI ALLEVAMENTO SULLO STATO DI BENESSERE DI TROTE IRIDEE VALUTATI MEDIANTE MONITORAGGIO DI PARAMETRI PLASMATICI E TISSUTALI

La sperimentazione aveva come obiettivo determinare lo stato di benessere di trote iridee (50±15 g) allevate in due vasche in cemento, con densità iniziali diversificate, tali da consentire il raggiungimento di carichi unitari finali rispettivamente di 40 kg/m³ (V1) e 20 kg/m³ (V2). Nel corso della fase di "ingrasso", il profilo ematochimico e i livelli di glic-
I geno epatico venivano monitorati ogni tre mesi, e confrontati con quelli di trote di pari età e peso allevate in condizioni extensive, in un invaso artificiale (VE).

I rilievi condotti hanno evidenziato differenze significative solo tra le trote allevate in vasca e quelle mantenute in condizioni extensive. Gli effetti a livello plasmatico si sono manifestati attraverso un aumento dei trigliceridi, del colesterolo totale e della fosfatasi alcalina nelle trote allevate in vasca; i livelli più elevati evidenziati nei pesci delle vasche V1 e V2 potrebbero attribuirsi, oltre che alle condizioni di allevamento intensivo, anche all'alimentazione bilanciata ed alle conseguenti modificazioni del metabolismo energetico. Le transaminasi (AST, ALT) risultarono significativamente maggiori in V1 e V2 limitatamente al prelievo primaverile. I valori di questi ultimi due parametri sono comunque rientrati nella media nei prelievi successivi lasciando supporre un avvenuto adattamento alle più severe condizioni di confinamento, tipiche dei bacini di ingrosso. Non sono invece emerse differenze apprezzabili tra i gruppi a confronto per proteine, glucosio, CK e LDH così come nel caso del glicogeno epatico.

Parole chiave: Trota iridea, Indicatori di benessere, Parametri ematochimici, Glicogeno epatico.

Introduction

After great development over the last two decades, the European fish culture, is currently moving toward a slower productive rhythm as a result of market saturation for salmon, rainbow trout, sea bream and sea bass and a new policy, aimed at reducing the environmental impact of intensive aquaculture. This strategy tends to promote the responsible management of fish farming and agrees perfectly with the welfare of the reared animals.

As for terrestrial species, in accordance with Directive 98/58 EC (OJ L 221 of 8/8/1998) on the protection of farmed animals, the European Union has for several years nominated a Standing Committee on reared animals, to develop guidelines on the welfare of the farmed aquatic organisms. Important associations involved in this Scientific Committee have published documents (FAWC, 1996; FSBI, 2002) concerning the welfare of fish, focusing on several aspects that have not yet been cleared up. Fish welfare has also been the subject of sessions at recent conferences organized by the European Aquaculture Society to stimulate the debate on the concept of welfare from different points of view (Kestin, 1994; Huntingford, 2002; Gornati et al., 2004). In addition, Fish Producer Associations and Great Distribution Retailers have adopted Codes of Conduct that include farmed fish welfare among the criterion, thus representing a primary goal to achieve since it has effects on yield and quality traits (Gregory, 1998; Huss et al., 2003). The welfare condition can be improved by reducing negative aspects directly related to practice and could improve the public perception of intensive aquaculture.

It is well known that farmed fish are often subjected to adverse stimuli causing acute (Pickering, 1981; Pottinger et al., 1999) or chronic (Sumpter, 1997; Montero et al., 1999) stress. External (environment) and internal (disease, metabolic unbalance) stressors are perceived by fish, which react with a primary neuroendocrine response represented by an increase in corticosteroids and catecholamines (Pickering, 1993). As a direct consequence of their high levels in circulation, a wide range of secondary stress responses are observed, such as the increase of blood glucose (Mazeaud et al., 1977; Pickering, 1981; Melotti et al., 1992) to which the decrease in hepatic glycogen is often associated. In order to face the increased demand of energy occurring in stress conditions (Barton and Iwama, 1991; Vijayan et al., 1994), hepatic glycogen is quickly converted to glucose. Hepatic glycogen is a metabolic indicator of secondary stress response (Begum and Vijayaraghavan, 1995; Shoemaker et al., 2003), but does not change when acute stress hits fish in good nutritional status (Barton et al., 1987).

The purpose of the present work is to determine the welfare status of rainbow trout intensively reared with different stocking densities. Because these fish could be submitted to adverse stimuli for a prolonged period of their life, we wanted to investigate chronic stress conditions by studying the general status of rainbow trout during the grow out phase.
Table 1. Proximate composition, energy content and fatty acid profile of the commercial diet (± SD).

| Component                        | Value (± SD)     |
|----------------------------------|-----------------|
| Dry matter %                     | 93.61 ± 1.24    |
| Crude protein % as it is         | 45.26 ± 4.07    |
| Crude fat %                      | 17.39 ± 0.81    |
| Ash %                            | 9.21 ± 1.25     |
| Crude fiber %                    | 1.70 ± 0.20     |
| N-free extract %                 | 20.04 ± 2.91    |
| Metabolizable energy MJ/kg DM    | 15.74 ± 1.30    |
| Fatty acid profile:              |                 |
| Saturated %                      | 32.71 ± 4.87    |
| Monounsaturated %                | 27.72 ± 0.75    |
| Polyunsaturated %                | 39.57 ± 4.82    |
| Total omega-3 %                  | 33.95 ± 5.33    |
| EPA %                            | 10.09 ± 1.54    |
| DPA %                            | 1.03 ± 0.05     |
| DHA %                            | 13.05 ± 0.90    |

Material and methods

The experimental protocol forecasted the comparison among 3 groups of rainbow trout of the same batch, size and age (4 months old), reared at different stocking density. In two small raceways (6x20x1 m) (V1, V2), located in a trout farm in central Italy supplied with well water, young rainbow trout were stocked at different densities so as to predict a final load of 40 kg/m³ (V1) and 20 kg/m³ (V2). Fish were fed extruded diets (Table 1) with a daily feeding level ranging during the trial from 0.3 to 1.2% b.w. according to the water temperature and fish size. Contemporarily, in an artificial reservoir (diameter 40 m, depth 3 m) (VE) supplied with well water, a small group of the same trout (95 fish) were introduced at a very low density. These trout received a limited quantity of the extruded feed (0.4% b.w. 3 times/week) in the first four months of the trial so as to maintain fish in natural conditions. After that time, VE feeding consisted only of trophic resources found in the environment. The most important data about the productive cycle are reported in Table 2.

Table 2. Main farming parameters of the trial.

| Parameter                        | V1         | V2         | VE         |
|----------------------------------|------------|------------|------------|
| Initial mean weight (±SD) g      | 50±15      | 50±15      | 50±15      |
| Initial stocking density n. fish/m³ | 88         | 38         | 95°        |
| Final stocking density kg/m³     | 40         | 20         | 85^        |
| N. of samplings                  | 4          | 4          | 4          |
| Length of the rearing cycle months | 14         | 14         | 14         |
| Water exchange l/sec/t           | 5          | 5          | -          |

°: total number; ^: kg/ha
Every three months, samplings were carried out contemporarily on the three groups of trout until commercial size was determined; they were made at the same hour of the day, for the different monitorings and for all the batches, in order to minimize circadian variations (Benneman, 1977). A total of five fish from each group were netted and anaesthetized individually. Blood was collected by heart puncture with a heparinized syringe; plasma was separated by centrifugation at 3000 rpm for 20 min, then frozen at –20 °C and stored for the subsequent assays.

The following plasmatic parameters were determined spectrophotometrically (Bergmeyer, 1974): glucose (GOD-PAP Trinder Method); total cholesterol (CHOD-POD Trinder Method); triglycerides (GPO colorimetric Method); total protein (Biurete colorimetric Method); LDH (lactate dehydrogenase) (UV optimized SCE Method); transaminases, such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT) (UV optimized IFCC Method); ALP (alkaline phosphatase) (DEA Method); CK (creatine kinase) (UV Method).

For the liver glycogen determinations, five fish (different from those used for blood sampling), were randomly netted from each batch and immediately sacrificed. Livers were dissected, weighed and frozen for glycogen analyses. The glycogen content was determined according to the Perry et al. method (1988) modified by Pavlidis et al. (2003). Samples were homogenated and digested in perchloric acid in order to extract glucose. The hydrolysis of glycogen to glucose was catalyzed by amyloglucosidase enzyme. Glucose levels obtained and read by spectrophotometer, were converted in milligrams of glycogen per gram of fresh liver tissue.

At the end of the trial, mean body weight and total length were recorded on 50 specimens from each batch. Condition factor (Beckman, 1948), hepatosomatic index (Moyes and West, 1995), weight gain and specific growth rate were also calculated.

Water quality parameters were monitored weekly in each batch and pH and total ammonia nitrogen were analysed in the lab following the standard procedures (A.P.H.A., 1989).

Data collected at the same seasonal sampling and at the end of the trial, have been submitted to one-way analysis of variance (ANOVA) (density of rearing) using the procedure of the Statistical Analysis System (SAS, 1989). Differences among the means were analysed by the Student-Newman-Keuls test.

**Results and discussion**

Water parameters (Table 3) were always perfectly compatible with the requirements of the reared species.

In Table 4 values of plasma parameters and liver glycogen are reported. Throughout all the time of the trial, triglycerides, total cholesterol and alkaline phosphatase resulted significantly higher in V1 and V2 trout, compared with VE batch; however, the maximum and minimum levels of these parameters always stayed within natural physiologic values. Concentrations lower than these (>40 mg/100 ml) are reported by Lee et al. (2003) in fish fed diets containing a high percentage of saturated fatty acids. In European sea bass, significant differences were found in plasma lipids using diets with and without fish oil supplementation (Lemaire et al., 1991). As regards alkaline phos-
Table 4. Plasmatic parameters and hepatic glycogen of rainbow trout reared at different densities and sampled every 3 months.

|                      | V1            | V2            | VE            | SE            |
|----------------------|---------------|---------------|---------------|---------------|
| **Triglycerids (mg/100 ml):** |               |               |               |               |
| Spring               | 206.0<sup>a</sup> | 189.9<sup>a</sup> | 121.0<sup>b</sup> | 16.44         |
| Summer               | 199.0<sup>a</sup> | 185.6<sup>a</sup> | 95.0<sup>b</sup>  | 23.07         |
| Autumn               | 215.0<sup>a</sup> | 198.4<sup>a</sup> | 116.0<sup>b</sup> | 24.54         |
| Winter               | 217.0<sup>a</sup> | 195.9<sup>a</sup> | 127.0<sup>b</sup> | 17.92         |
| **Total cholesterol (mg/100 ml):** |               |               |               |               |
| Spring               | 225.0<sup>a</sup> | 202.0<sup>a</sup> | 125.0<sup>b</sup> | 36.14         |
| Summer               | 170.0<sup>a</sup> | 171.2<sup>a</sup> | 94.0<sup>b</sup>  | 13.54         |
| Autumn               | 167.0<sup>a</sup> | 162.0<sup>a</sup> | 93.8<sup>b</sup>  | 18.26         |
| Winter               | 213.0<sup>a</sup> | 211.6<sup>a</sup> | 95.0<sup>b</sup>  | 27.70         |
| **Total protein (U/l):** |               |               |               |               |
| Spring               | 3.06          | 3.04          | 3.08          | 0.30          |
| Summer               | 3.16          | 3.14          | 2.80          | 0.37          |
| Autumn               | 3.00          | 3.08          | 2.82          | 0.53          |
| Winter               | 2.98          | 2.85          | 2.52          | 0.51          |
| **LDH (U/l):**       |               |               |               |               |
| Spring               | 52.40         | 48.20         | 44.80         | 26.56         |
| Summer               | 45.40         | 44.00         | 43.02         | 16.27         |
| Autumn               | 45.60         | 45.20         | 42.00         | 18.11         |
| Winter               | 52.40         | 49.00         | 48.90         | 23.24         |
| **ALP (U/l):**       |               |               |               |               |
| Spring               | 60.40         | 59.00         | 46.01<sup>b</sup> | 5.39          |
| Summer               | 64.02<sup>a</sup> | 61.40<sup>a</sup> | 44.80<sup>b</sup> | 6.20          |
| Autumn               | 100.80<sup>a</sup> | 99.60<sup>a</sup> | 60.81<sup>b</sup> | 16.03         |
| Winter               | 71.79<sup>a</sup> | 75.20<sup>a</sup> | 50.02<sup>b</sup> | 11.05         |
| **CK (U/l):**        |               |               |               |               |
| Spring               | 50.60         | 49.20         | 48.80         | 10.47         |
| Summer               | 45.20         | 43.40         | 44.00         | 8.07          |
| Autumn               | 45.80         | 48.00         | 41.82         | 20.97         |
| Winter               | 60.80         | 60.62         | 59.07         | 16.65         |
| **AST (U/l):**       |               |               |               |               |
| Spring               | 52.45<sup>a</sup> | 48.13<sup>a</sup> | 29.00<sup>b</sup> | 9.42          |
| Summer               | 38.80         | 39.20         | 37.29         | 19.04         |
| Autumn               | 40.20         | 43.20         | 42.20         | 10.91         |
| Winter               | 40.00         | 42.00         | 38.20         | 13.36         |
| **ALT (U/l):**       |               |               |               |               |
| Spring               | 43.20<sup>a</sup> | 42.11<sup>a</sup> | 23.00<sup>b</sup> | 4.59          |
| Summer               | 25.20         | 26.59         | 24.80         | 5.88          |
| Autumn               | 21.80         | 22.81         | 20.60         | 5.75          |
| Winter               | 21.40         | 23.80         | 22.12         | 4.56          |
| **Glucose (mg/100 ml):** |               |               |               |               |
| Spring               | 68.80         | 65.90         | 48.20         | 16.84         |
| Summer               | 61.40         | 60.80         | 46.00         | 26.53         |
| Autumn               | 70.00         | 60.99         | 67.10         | 18.38         |
| Winter               | 70.40         | 72.80         | 74.80         | 25.27         |
| **Hepatic glycogen (mg/g liver):** |               |               |               |               |
| Spring               | 258.4         | 244.6         | 200.8         | 104.89        |
| Summer               | 237.4         | 232.0         | 220.4         | 67.14         |
| Autumn               | 256.8         | 262.6         | 233.5         | 75.78         |
| Winter               | 288.0         | 255.3         | 221.2         | 83.55         |

<sup>a, b</sup> P<0.05
Table 5. Biometric parameters and indices of fish sampled at the end of the trial.

| Parameter                  | V1        | V2        | VE        | SE        |
|----------------------------|-----------|-----------|-----------|-----------|
| Final weight g             | 450.87a   | 540.11a   | 358.20b   | 58.83     |
| Final length cm            | 33.69a    | 34.80a    | 30.37b    | 9.29      |
| Daily weight gain g/d      | 0.94a     | 1.15a     | 0.72b     | 0.18      |
| Specific growth rate       | 0.52a     | 0.56a     | 0.46b     | 0.04      |
| Condition index            | 1.34a     | 1.42a     | 1.11b     | 0.06      |
| Hepatosomatic index        | 1.66b     | 2.01a     | 1.89c     | 0.04      |

* a, b, c: P<0.05

Phosphatase, the activity of this enzyme appeared stable in all the groups in agreement with previous findings (Heath, 1987) who remarked the role of this membrane enzyme related to physiological status of gut and bile ducts.

Plasma total protein, CK and LDH concentrations, due to the high variability, did not show significant differences. Transaminases (AST, ALT) fish levels were normal for this species (Miller et al., 1983). In the two groups of farmed fish a noticeable increase was found only at the first sampling, carried out in spring, and this difference became not significant with the progression of the trial. As regards plasma glucose, no statistical difference emerged among the groups with a high homogeneity over time noted in the specimens reared in V1 and V2 tanks.

In terms of hepatic glycogen no significant difference was observed among treatments. There are also different works (Barton and Iwama, 1991; Korshon et al., 1994; Begum and Vijayaraghavan, 1995) stating this situation and indicating that good energetic stores, such as glycogen at hepatic level (Reddy and Leatherland, 1994), are able to withstand stress situations and thus to support rearing at high stocking density (Vijayan et al., 1990).

As regards biometric parameters and indices, important differences were noted among V1, V2 and VE batch (Table 5). Final mean weight, daily weight gain and specific growth rate were significantly higher in the intensive groups compared to the extensive one. A similar result was obtained in terms of mean length; trout held in the natural reservoir presented a condition index lower than fish in tanks. This situation could be explained with the reduced availability of feed for these animals. Considering the hepatosomatic index, marked differences were also found among the three batches. In a recent review by Ellis et al. (2002), to establish if the stocking density affects the welfare status of rainbow trout, condition factor and hepatosomatic index are considered among the main stress indicators and show changes at the increase of stocking density. Numerous studies have investigated the effects of density on productivity but the findings are not always in agreement. In brook charr, Hardy and Audet (1990) observed that growth performances were not affected by density and interpreted this as an example of good application of rearing management and high water quality. When these conditions are not respected, the increasing stocking density leads to a reduced food consumption as a consequence of deterioration in water quality, overcrowding or adverse social interactions (Kebus et al., 1992; Moyes and West, 1995; Ellis et al., 2002).

Conclusions

The results of the present work show significant differences only for some plasma parameters in rainbow trout reared in raceways compared with those held in extensive conditions. Plasma parameter variations were evident in terms of triglycerides, total cholesterol and alkaline phosphatase in fish reared in tanks. The highest levels constantly recorded in V1 and V2 tank could be attributed mostly to the balanced feeding and consequent modifications of energetic metabolism.
(Pfeffer, 1995) and, in a small part, to the high density of the intensive rearing conditions. Transaminases (AST, ALT) increased at only the first sampling; afterwards, the activity of these two enzymes returned to the normal level suggesting a capacity of the rainbow trout to adaptation under the most severe conditions of confinement as sustained by other fish species (Lemaire et al., 1991; Montero et al., 1999). No differences among groups were shown with respect to total protein, glucose, CK and LDH. A similar situation was also related to glycogen content in liver.

In conclusion, it could be sustained that rainbow trout in the rearing conditions tested in this study do not seem to be affected by chronic stress, although levels of some plasma parameters resulted higher compared to animals held in extensive conditions.

It could thus be considered possible that changes of plasma parameters can be evident in rainbow trout at stocking densities higher than those tested in our trial. Papoutsoglou et al. (1987) monitored plasma lipids and glucose at more elevated stocking densities than those tested by us; at the end of a full rearing period, they noted a tendency to decrease with an increase in fish density of around 60 kg/m³. In other species, densities around 60 kg/m³ have shown adverse effects on plasma metabolites related to hepatic function and energy stores which lead to compromised fish health (Hardy and Audet, 1990; Begum and Vijayaraghavan, 1995; Shoemaker et al., 2003).

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