Lipid accumulation in rainbow trout (Oncorhynchus mykiss) muscle cells, reared in superintensive system

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Summary
In the present study we approached the problem of lipid accumulation in rainbow trout (Oncorhynchus mykiss) reared in superintensive system, at 50 kg/m³ density. If in farmed animals, lipid deposits are located visceral, subcutaneous or intramuscular, in rainbow trout we noticed their arrangement is located within the muscle fiber. Depending on the environmental conditions and food intake, these accumulations of fat exert increasing pressure on the muscle fiber, which gradually atrophies, and finally become the exclusive cellular lipid content. Muscle fiber plasmalemma remains intact, even if the cell volume increases and muscle fiber is replaced by lipid deposits. This phenomenon has been observed in a control group of rainbow trout (reared in classical system), but lipid accumulation process is more discreet and was reported just near the connective septa. In the group reared in superintensive system, lipid accumulation had sometimes dramatic aspects, meaning they appear as sac-shaped deposits of fat, bounded by endomysium. The consequence of this process is the degradation of rainbow trout meat quality, which calls into question the capability of superintensive growth systems to develope high quality products.

Introduction
The implementation of superintensive systems in aquaculture is the result of the growing demand in the market of fishery products. Increased consumption of fish meat is on the one hand due to global population growth and, on the other hand is the result of awareness regarding the beneficial effects of fisheries on human health (Byelashov & Griffin, 2014). These superintensive systems, are characterized by higher productivity compared to conventional farming systems (De Ionno et al., 2006; Martins et al., 2010). Production cycles are much shorter, slaughter yields are superior to conventional farming systems, feed conversion ratio is very low and the number of employees needed for such farms is smaller. Inevitable, it appears this question: what is wrong with this farming systems?

Making an analogy regarding this fish farming systems and industrial scale farming of other species, such as birds, pigs, cattle, etc., we noticed a common trait – a lower quality of meat (Lebret, 2008; Kuźniacka et al., 2014). In most species, the factors leading to a depreciation of meat quality are determined by the welfare (Grandin, 2014). Factors influencing meat quality of rainbow trout (Oncorhynchus mykiss) reared in superintensive systems are numerous, among we mention: physico-chemical parameters of...
water that are artificially maintained at optimal levels, with implications on metabolic rate (very high); high growth density, which leads to the increasing of reactive oxygen species – ROS - (oxidative stress); lack of space for swimming, and unidirectional swimming in circular tanks (which lead to uneven development of muscle segments), having direct implications in red muscle atrophy processes and in the ability to swim (Vranic et al., 2011; Lupoae et al., 2011; Karahmet et al., 2014). Regarding histological study of fish muscle, most researches focused on determining the smoothness of muscle fibers (Stickland, 1983; Nistor et al., 2013). This issue should be profoundly studied, due to the highly emphasized polymorphism in fish muscle cells. Previous research has shown an increase in the amount of fat in the fish meat reared at high densities (Cocan & Mireșan, 2011). Very little has been studied on these lipid storage mechanism in fish in general, and especially in rainbow trout. If in the case of farm animals, lipid deposits are located visceral, subcutaneous or intermuscular, then in rainbow trout we observed that lipid accumulation takes place inside the muscle fiber. The phenomenon is even more emphasized as the maintenance conditions allow this, in certain situations fat deposits gradually replace muscle fibers, to disposal.

Materials and methods

Rainbow trout (Oncorhynchus mykiss) specimens studied had common genetic origins. The control group was reared at Fiad trout farm, Bistrița-Năsăud County (classic rearing system) and the experimental group was reared in an experimental superintensive farm in Cluj-Napoca, Cluj County, at high density (50 kg/m3). Administered feed and feeding rate was identical in both groups. For the histological studies were randomly sampled 10 rainbow trout specimens of each group. From the dorsal region were sampled fragments of 5 mm thickness, which intercepted the deep and superficial layers of muscles in the area of choice. After fixation in 15% formol solution for 5 days, the samples were dehydrated in ethylic alcohol, cleared in 1-Butanol, embedded in paraffin, sectioned at 5 µm thick, stained using Goldner's Trichrome method, examined under optical microscope (Olympus BX 41 ) and were captured digital images (Olympus E 330). The captured images were then processed in Adobe Photoshop CS Image Editor 2 V. 9.0.

Results and discussions

Somatic musculature of rainbow trout is formed of skeletal muscle cells, having a high variability of thickness. The differences of the diameter are extremely large from a muscle cell to another. Muscle cells polymorphism is not the same for all groups of muscles. Superficial muscles present a low polymorphism compared to deeper muscle layers.

In the control group, superficial muscles are formed by muscle cells of medium thickness with relatively small differences in diameter and compact layout (no large gaps are formed between them). Superficial muscle layer is separated from the depth layer by a connective septum (perimysium) and muscle cells in the deeper layers differ from those of the surface layer, both in terms of polymorphism and tinctorial affinity (Fig. 1).

![Fig. 1. Muscle layers aspects. Red arrows – superficial layer; blue arrows – deeper layer; black arrows – perimysium.](image-url)
Muscle cells in the deeper layers do not appear compact arranged as the superficial muscles, between them being present relatively large gaps. Most of the deeper layers of muscle cells are devoid of content (it seems that the spaces or gaps were occupied by liquid substances extracted during histological processing).

Near connective septs (perimysium), were highlighted cells having degenerative aspects, of different intensities, evidenced by the presence of inter myofibrillar spaces and having tendency to deposit a granular material in the space between the endomysium and muscle cells. In some muscle cells the process is more advanced, in sense that the interfibrillar edema is less pronounced and in addition appears myofibrillar lysis areas. They generate spaces of different sizes where myofibrils is missing.

The granular material disposed under endomysium (between it and the muscle cells) is present in large quantities in a small number of muscle cells. By accumulation of an amount of increasingly higher granular material, a gradual atrophy of muscle cells appears. Does not seem a pathological state, but a storage of substances that we consider to be of lipid nature. Seems a way to deposit excess fat. We mention that this process comprises a small number of cells arranged mostly near connective septs (perimysium). By aspect is seems a normal way of storing lipids (fattening). In the control group, the fattening process is discreet, but its presence, even at this level demonstrates that fish have good phisyological state (Fig. 2).

The situation is identical both to the experimental group and to the control group, in terms of structure and appearance of superficial muscles, that appear here, formed from muscle cells with average diameter, arranged side by side (without large spaces between them), and do not show things that betray activities storage of substances (of any kind). This superficial muscles do not have storage activities of substances (of any kind).

In the profound muscles, storage processes reported in controls, are also present, but the processes have great intensity and cover large areas. Lipid storage workflows of appreciable intensity, allows their dynamic tracking. There are situations where lipid deposits accumulate in increasingly higher amounts, between endomysium and muscle cells, which results in significant expansion on one side of this gap, and on the other hand, exercising a compression on the muscle cell, this causing a progressive atrophy (Fig. 3).

By increasing the amount of lipids deposited, compression atrophy of muscle cells is more pronounced, so that they have only vestigial aspect (Fig. 4). Some muscle cells disappeared, and in their place were formed sac-shaped lipid deposits, bounded by endomysium. Their sizes are much larger than those of the muscle cell, of which they were formed (Fig. 5).
By developing and enhancing these processes of lipid storage, the number of muscle cells that disappear and are replaced by sac-shaped lipid deposits, their number being very high in some cases. The starting point for the formation of these huge deposits of lipid is from the connective septs (perimysium) so that muscle cells in the vicinity of large areas disappear, and are replaced by fat. If food intake of fish and rearing conditions permit, the replacement of muscle cells with fat deposits intensifies, tending to cover large areas.

**Conclusions**

The fish reared in classic system showed a slight accumulation of lipid in interfibrillar spaces, and also a discrete fat load of cells located in the peripheral fascicles, and rarely in their depth.

The fish reared in superintensive system, a large number of muscle cells, both in the periphery and in the depth of fascicles, load gradually until the former muscle cell lipid turns into a real sac of fat.

Over forcing feeding of the rainbow trout reared in superintensive systems does not increase muscle mass, but replacing muscle tissue to fat tissue, which results in a drastic decrease in rainbow trout meat quality.

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