GLYCOLYTIC AND OXIDATIVE ACTIVITIES OF BULLFROG (*RANA CATESBEIANA*) SKELETAL MUSCLES DURING POST-METAMORPHOSIS GROWTH

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Background

The biochemical characteristic of skeletal muscle fibers is an important factor influencing many peri- and post-mortal biochemical process and thereby meat quality (KLONT et al., 1998), with direct effects on processing and storage. The predominant fiber type of skeletal muscle can affect meat tenderness, texture, juiceness, flavor, color and carcass yield (ABERLE et al., 2000). The muscle fiber type may be classified by histochemical and, or, biochemical analysis. Biochemical analysis gives a better indication of aerobic and anaerobic muscle capacity and thus has been used to evaluate muscle metabolic properties by measuring enzymatic activities (ESSÉN et al., 1980). Citrate synthase (CS; EC 4.1.3.7) and lactate dehydrogenase (LDH; EC 1.1.1.27) are frequently used as, respectively, the oxidative and glycolytic marker enzymes. OGATA & MORI (1963) classified the muscle fibers of reptiles and amphibians into three types, on the basis of their diameter and activity of oxidative enzymes. Red fibers show higher activity of oxidative enzymes, the white fiber shows a lower activity and the medium fibers displays an intermediate enzymatic reaction. In amphibians locomotory muscles, PUTNAM & BENNETT (1983) found that glycolitic white fibers occupies 80 to 90% of the area of the bulk region, with the remainder composed of medium fiber (glycolytic and oxidative characteristics). In *Rana pipiens* muscles, red fibers (oxidative) generally accounted for less than 1% of the cross sectional area. Although bullfrog meat also has a visual apearance suggesting a wide predominance of white fibers, MOURA (2000) reported, depending on the stunning procedure, an 8 to 12 hours delay in Rigor Mortis development in this amphibian. This is a very extensive time for Rigor development in glycolytic muscles and may indicate fiber differentiation from white (glycolytic) to red (oxidative) which may be influenced by its post-metamorphosis growth (liveweight) , specially due to exercise and training effect (LAWRIE, 1985) of jumping activity. However, the literature concerning most of the domestic animals indicates that fiber differentiation occurs the otherway around (from red to white) as animal ages (KARLSON et al., 1999; ABERLE et al., 2000) even though LAWRIE (1985) establishes that the cytochrome oxidase activity at birth is only 8 per cent of its adult value. However, so far as we know there is no literature indication on the effect of age on amphibian muscular metabolic changes.

Objectives

The aim of this paper was to evaluate the oxidative and glycolytic capacity of bullfrog skeletal muscles fibers and their differentiation during post-metamorphosis growth, by means of evaluating the relationship between LDH and CS activities.

Methods

Six metamorphosed animals of each selected weight (100, 150, 200, 250 and 300 ± 5 g) was obtained from UFV Anfrigranja frog farming system, developed by LIMA & AGOSTINHO (1988). **Slaughtering**: The selected frogs were identified, weighed and maintained off feed for 48 hours before slaughtering. After stunning by immersion in water and ice (1:1) for 15 minutes, the frogs were bled by cutting the cardiac veins, and their carcasses eviscerated, washed and bath chilled in a tank containing water and ice. The *gastrocnemius* muscles were removed and one of them was immediately submitted to the enzyme extraction procedure. The other muscle was submitted to pigment analysis. **Enzyme extraction:** Each muscle was minced in a Marconi sample homogenizer, weighted and homogenized in 19 times their volume in a buffer (pH 7,4) containing 2 mM EDTA and 175 mM KCl. Homogenates were frozen and thawed three times and then centrifuged at 3000 *g* (4°C) for 10 minutes (PUTNAM & BENNETT, 1983). Supernatants were used for enzymatic analysis. **Enzyme Activities**: The lactate dehydrogenase (LDH) assay followed the procedure of BERGMEYER & BERNT (1974) and citrate synthase (CS) followed the procedure of SRERE (1969). The protein content of the supernatants was determined using the biuret assay. All enzyme activities were expressed in terms of micromoles of product per minute and gram of muscle (U/g muscle) or micromoles of product per minute and gram of protein (U/g protein). **Pigment Analysis**: The pigment content was evaluated following the hematin procedure (HORNSEY, 1956) measured at 640 nm. The achromatic attenuance of light at 730 nm (KRZYWICKI, 1979) was used to avoid turbidity interference.

Results and Discussion

The results show that both the glycolytic (Figure 1A) and oxidative (Figure 1B) metabolism of bullfrog *gastrocnemius* incresea with age (liveweight). However, it becomes increasingly more oxidadtive (Figure 1C) with age (lower LDH / CS ratio) which is accompanied by an increase in total pigment concentration (Figure 1D). Increase in oxidative metabolism and pigment concentration with age is in agreement with the fiber differentiation trend described by LAWRIE (1985) in pork up to one year of age and which is related to an increase in muscle myoglobin concentration. It is also supported by Suzuki and Cassens (1980) in pigs from birth to 8 weeks of age (KARLSON et al., 1999). However, this trend is in opposition to those reported by ABERLE et al. (2000) and most of the research reviewd by KARLSON et al. (1999) in pork. The increase in oxidative metabolism (lower LDH / CS ratio) in bullfrog *gastrocnemius* muscle may reflect the training effect reported by LAWRIE (1985) and may be due to the fact that frog raised in capitivity become less active, lowering its jumping activity as the need to run after food and from predators are minimized. In addition, heavier frogs dominate theire mates and prevail with respect to food access in the farm. In this way as it ages the bullfrog so raised becomes a slower jumper and the *gastrocnemius* muscle is less required as well as its need for an immediate energy source. CHOI & PARK (1996) showed that toads, slow jumping frogs, compared to *Rana*, fast jumping frogs, have a greater CS / LDH ratio in *gastrocnemius* muscle. Despite the above discussion, these changes do not seem enough to change muscle fiber classification and alter post-mortem rigor development as, though CS activity increases proportionally more, the nominal increase in LDH activity is much greater (Figures 1A and 1B). In this way, the high RM time found by MOURA (2000) in bullfrogs is most probably influenced by the mebalolic and physiological characteristics of frog muscles, such as its capacity to metabolize lactate (FOURNIER & GUDERLEY, 1992; FOURNIER et al., 1994), utilize alanine as an energy substrate (STOREY & STOREY 1986), as well as posses a high capacity to absorb atmospheric oxigen (BROWN JR., 1964).

Conclusions

Although the gastrocnemius muscle of farm raised bullfrog becomes increasingly more oxidative (higher CS/LDH activity ratio), the muscle fiber are predominantly white, with much higher LDH activity than CS activity.

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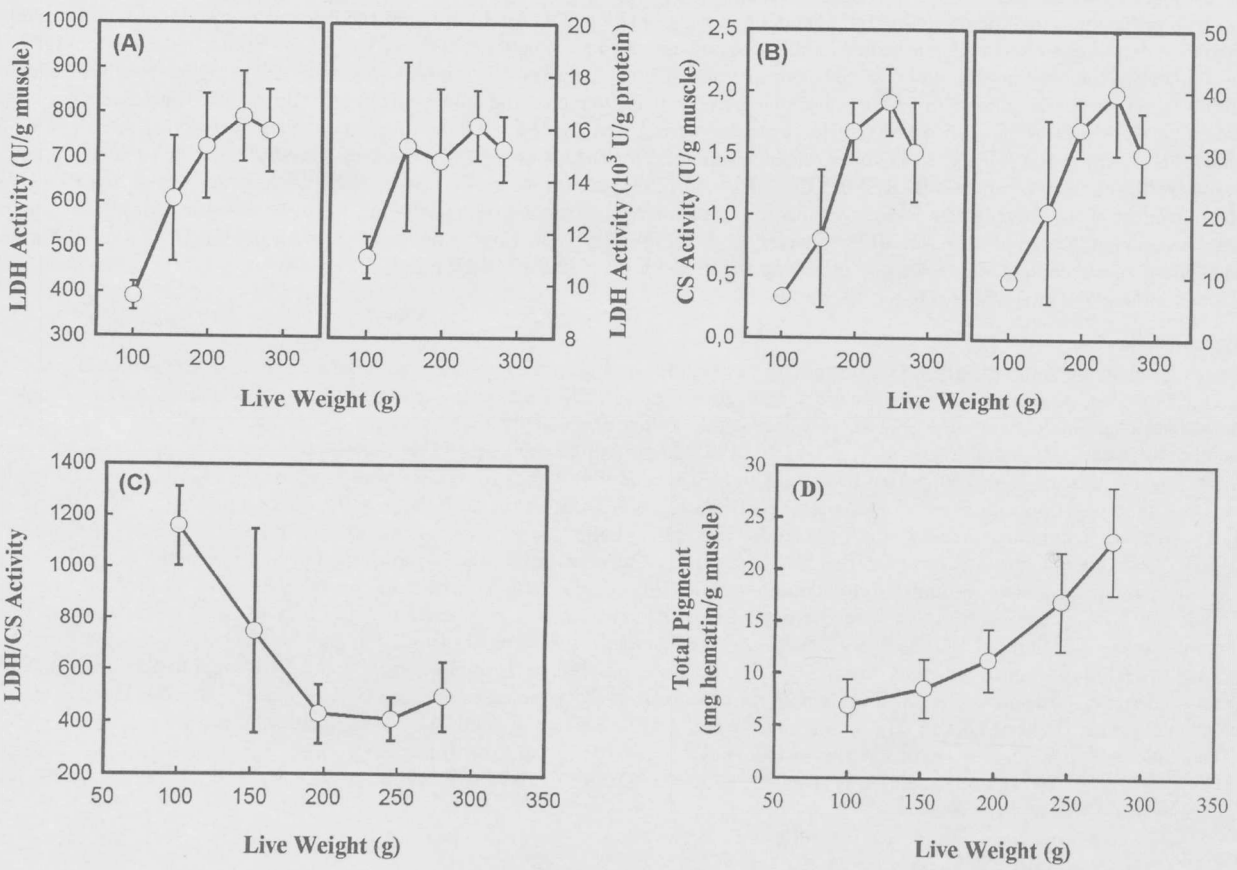

Figure 1. Enzymatic activities and pigment content in bullfrog *gastrocnemius* muscle during growth: (A) LDH activity; (B) CS activity; (C) Relationship between LDH and CS activity; and (D) Total pigment content.