

**EFFECTS OF β -AGONIST FEEDING ON RAINBOW TROUT
(*ONCORHYNCHUS MYKISS*) MUSCLE GROWTH
AND MYOSATELLITE CELLS**

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EXTENDED ABSTRACT ONLY- DO NOT CITE

The adrenergic system, characterized by the catecholamine hormones adrenaline and noradrenaline, integrates and modulates many aspects of vertebrate, including fish, metabolism. β_2 -Adrenergic agonists (β_2 -AA; synthetic catecholamine analogues signaling through the β_2 -adrenergic receptor) increase muscle growth in mammals (Maltin et al., 1992) and act as repartitioning agents by re-directing nutrients from adipose tissue to muscle (Roberts and McGeachie, 1992). β_2 -Agonists also increase weight gain, feed utilization, lean body mass and protein accretion in mammals.

In this study, we examined the effects of ractopamine (RACT) and clenbuterol (CLEN), two β_2 -adrenergic agonists of the phenethanolamine group, on both rainbow trout muscle growth and myosatellite cell proliferation and differentiation. Currently, ractopamine is commercially used in swine farming, and has previously been tested in rainbow trout (Vandenberg and Moccia, 1998) and channel catfish (*Ictalurus punctatus*) (Mustin and Lovell, 1993) where it has demonstrated lower effects than reported in other vertebrates. Clenbuterol increases myosatellite proliferation in mice (Roberts and McGeachie, 1992) and muscle fiber hypertrophy in Lister rats (Maltin et al., 1992), but its effects on fish myosatellite cells and muscle growth remain unknown.

Rainbow trout (80 fish per 110-115 L tank) were fed the following three experimental diets: commercial diet sprayed with 10 ppm ractopamine, 10 ppm clenbuterol or carrier (sham) for 10 to 12 weeks. Fish were ~ 6 g at the beginning of the experiment and ~ 30g at 12 weeks. After exposure, 12 fish per group were sacrificed and blood, liver, white and red muscle, gut and kidney were sampled for metabolic enzyme and metabolite assays; liver and visceral lipid were removed and weighed to calculate hepatosomatic index (HSI) and lipid somatic index (LSI). Ten additional fish per group were used for BrdU (bromo-deoxy-uridine) injection to characterize *in situ* myosatellite cell proliferation. The remaining fish were used for myosatellite cell extraction according to Fauconneau and Paboeuf (2000) to characterize *in vitro* proliferation and differentiation. Data were analyzed with ANOVA followed by Tukey-Kramer test and T-test with the JUMP program.

Clenbuterol- and RACT-treated fish had significantly higher condition factor (CF) compared with sham fish (Table 1) and RACT-treated fish had a lower visceral lipid content (Table 1). No significant differences were found in HSI

Table 1: Condition factor (mean \pm SE) at the beginning of the experiment (CFi) and at the end of the exposure (CFe), hepatosomatic index (HSIe) (mean \pm SE) and lipid somatic index (LSIe) (mean \pm SE) at the end of the exposure; n represents the number of fish. CF, HIS and LSI have been calculated respectively by the following equations [(weight \cdot length⁻³) \times 100, (liver weight \cdot total weight⁻¹) \times 100 and (weight of abdominal cavity lipids \cdot total weight⁻¹) \times 100. **, indicates significant differences compared to the sham ($\alpha = 0.05$)

Treatment	n	CFi	CFe	HSIe	LSIe
SHAM	12	1.55 \pm 0.05	1.11 \pm 0.02	1.15 \pm 0.19	1.84 \pm 0.09
RACT	12	1.23 \pm 0.05	1.35 \pm 0.04 **	1.39 \pm 0.09	1.42 \pm 0.10 **
CLEN	12	1.34 \pm 0.16	1.42 \pm 0.06 **	1.12 \pm 0.13	1.65 \pm 0.11

between treatments. Preliminary results indicate that the activities of alanine aminotransferase (ALT), pyruvate kinase (PK) and isocitrate dehydrogenase (IDH) in the liver were higher in fish treated with 10 ppm RACT compared with sham fish (Table 2). There were no differences in phosphoenolpyruvate

Table 2: Specific activity ($\mu\text{mol}\cdot\text{min}^{-1}\cdot\text{mg}^{-1}$ protein) of pyruvate kinase (PK), alanine aminotransferase (ALT), aspartate aminotransferase (AST), glucose-6-phosphate dehydrogenase (G6PDH), malic enzyme (ME) and isocitrate dehydrogenase (IDH) in liver (mean \pm SE, n = 8). **, indicates significant differences compared to the sham ($\alpha = 0.05$); *, indicates significant differences compared to the sham ($\alpha = 0.1$)

Treatment	SHAM	CLEN	RACT
PK	1.60 \pm 0.21	1.69 \pm 0.22	2.19 \pm 0.19
ALT	1.71 \pm 0.17	1.98 \pm 0.22	2.65 \pm 0.18 **
AST	3.39 \pm 0.29	2.67 \pm 0.13 **	3.54 \pm 0.19
G6PDH	2.02 \pm 0.15	1.41 \pm 0.10 **	1.93 \pm 0.14
ME	0.46 \pm 0.09	0.39 \pm 0.02 **	0.47 \pm 0.05
IDH	1.05 \pm 0.06	0.87 \pm 0.07 *	1.25 \pm 0.07 *

carboxykinase (PEPCK), aspartate aminotransferase (AST), glutamate dehydrogenase (GDH), lactate dehydrogenase (LDH), malate dehydrogenase (MDH), glucose-6-phosphate (G6PDH), or malic enzyme (ME) activities in the liver between sham and RACT-treated fish. The activities of AST, G6PDH, ME and IDH in the liver were lower in fish treated with CLEN compared with sham fish (Table 2). There were no differences between sham and CLEN-treated fish for LDH, MDH, GDH, ALT, PEPCK and PK in the liver. Extraction yield of myosatellite cells were 2.9×10^4 for the sham group, and 1.2×10^5 and 6.4×10^4 cells $\cdot\text{g}^{-1}$ muscle for the RACT- and CLEN-treated fish, respectively.

The higher CF and myosatellite cell extraction yield suggest that both β_2 -AAs administrated to rainbow trout for 10 to 12 weeks significantly increase muscle growth. The differences seen in the action of RACT and CLEN on liver metabolic enzymes suggest a different mode of action of those two β_2 -AAs. Ractopamine treatment increased AST, an enzyme implicated in protein metabolism and PK, a glycolytic enzyme, whereas CLEN treatment decreased G6PDH, ME and IDH, three enzymes implicated in lipid synthesis.

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