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Effects of Dietary Salbutamol on Growth and Body Composition of Rainbow Trout (*Oncorhynchus mykiss Walbaum*). (*Oncorhynchus mykiss Walbaum*)

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ABSTRACT

Salbutamol is a beta adrenergic agonist which reduces carcass fat and increases muscle mass and feed conversion efficiency in pigs. In the present study, the effects of dietary salbutamol at 20 ppm on growth, feed conversion ratio, carcass recovery, visceral organ weight, and whole carcass composition were studied. Eighteen months-old rainbow trout (initial weight 324 g) in 2*3 completely randomized design were fed either basal diet or basal diet + 20 ppm salbutamol diet for four weeks. Growth performance was measured weekly. At the end of the four week feeding period, fish were killed, eviscerated and whole carcasses were analyzed for protein, fat and ash. Dietary salbutamol had no adverse effect on fish mortality, health or feed intake. Dietary salbutamol had no significant ($p>0.05$) effect on growth performance and feed conversion rstio of rainbow trout but significantly increased ($p<0.01$) the carcass recovery. Internal organ weights such as liver, heart, gonads and viscero-somatic index and hepato-somatic index were also not affected by salbutamol. Interestingly, 20 ppm dietary salbutamol significantly ($p<0.01$) increased the weight of the kidney possibly, due to increased metabolic load on kidney and blood flow to the kidney. Whole carcass contents of protein, fat and ash of the fish showed no significant difference between treatments and clearly reflected the normal allometric growth and body composition. It was concluded that dietary salbutamol at 20 ppm level had no repartitioning effect in growing rainbow trout. However it is suggested to study the effects of salbutamol at various doses in more mature rainbow trout.

Key words : Rainbow trout, Beta agonists, Salbutamol, growth, Carcass composition.

INTRODUCTION

Aquaculture on world wide basis is now a profitable but competitive industry. High growth rate and low recurrent cost determine the productivity and the profitability of the operation. In intensive aquacultural systems where feed cost can be as high as 60% of the recurrent cost, high growth rate and efficiency of feed utilization by fish are of paramount importance in increasing productivity and profitability. From consumer's point of view, quality of animal product, particularly the carcass fat content has become a serious concern due to health reasons.

Various techniques have been used to increase the growth rate, feed efficiency and carcass quality, mainly in terrestrial animals. During last 25 years beta adrenergic agonists such as clenbuterol, cimaterol, ractopamine, L644, 969 and salbutamol have been intensively studied as a potential candidate for manipulating growth and carcass composition, mainly in terrestrial farm animals (Beerman *et al.*, 1987; Sota *et al.*, 1995; Kim *et al.*, 1995). It is generally accepted that these beta agonists change the carcass composition by increasing the skeletal muscle protein content while

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reducing fat content (Reviewed by Moloney *et al.*, 1992; NRC 1994; Moody *et al.*, 2000). In some cases growth and feed efficiency have also improved. Since these compounds redirect the nutrients away from fat deposition towards muscle hypertrophy, they are termed repartitioning agents. The major advantage of beta agonists is, unlike somatotropins, they are orally active and thus can be given with feeds. Until recently, the use of beta agonists had been banned in food industry. In 2001, two beta agonists namely zilpaterol and ractopamine have been cleared for food animal industry in several countries, including the USA.

Although beta agonists have been extensively studied in terrestrial farm animals, only few studies have been done with fish. Three research groups (Mustin and Lovell, 1993; 1995; Webster *et al.*, 1995 and Vandenberg and Moccia, 1998) have studied the effects of ractopamine and L644, 969 in channel catfish and rainbow trout. Available literature suggests that dietary beta agonists are not as effective in fish as in terrestrial animals (Atapattu, 2001). Channel catfish have found to be more sensitive to dietary beta agonist than rainbow trout. Salbutamol is a beta 2 adrenergic agonist that has been found to be effective in pigs. Rainbow trout is among the most widely and intensively reared aquacultural species. The present study investigated the effects of dietary salbutamol at 20 ppm on the growth, body composition and organ weight of young rainbow trout.

MATERIALS AND METHODS

A basal diet based on hoki meal (*Macruronus novaezealandiae*) and fish meal was formulated. The ingredients and nutrient composition of the basal diet are given in Table 1. Salbutamol was incorporated at a rate of 20 ppm into the basal diet. Following mixing, the diets were cold pelleted (65^0 C).

Animal and experimental design

The experiment followed a completely randomized design with two treatments (basal diet or basal diet + 20 ppm salbutamol) assigned to six concrete tanks (1.85*1.0m). Water level was maintained at 0.7 m. Each tank had one water inlet and one water outlet and was supplied with high quality water at a constant rate of 80 l/min. Each treatment was replicated three times. Fish were allocated into tanks so that between tank weight vitiation was minimum. Each replicate had 18 fish at the beginning of the acclimatization period. Fish were acclimatized for four weeks. During the accalatization period fish were fed basal diet (1.5% body weight). On the 29^{th} day of the acclimatization period, fish were lightly anaesthetized with Aqui_S (Fish Transport System, Lower Hut, New Zealand) and individually weighed but fish were not individually identified. Therefore, per tank fish weight was taken. Three fish from each tank was randomly captured. These fish were killed and organ weights (liver, heart and kidney) were taken. Carcasses were stored at -20 ^{0} C for later laboratory analysis for carcass protein, fat and ash. Remaining 90 (in six tanks) fish were fed basal or basal + 20 ppm salbutamol diet for 28 days. The fish were subjected to 12:12 h light:dark cycle. Water temperature varied from 9-11 ^{0}C (mean 10.3 ^{0}C).

Feeding method

Weekly feed allowance was calculated as 3% of the initial body weight. Daily allowance was fed twice a day; at 0830 and 1600 hours. Tanks were cleaned before each feeding. Fish were hand fed to satiety. Fish were fed until they stop actively seeking the food. Refused feed was collected using a gauze cloth attach to the outlet of a manual siphon. The refused feed were dried at 100 ^{0}C for 24 hours and weighed.

Fresh feed samples were also dried and the dry weight was calculated. On these data weight of the refused feed was computed.

Fish were off the feed on the 29th day of the feeding trial. On day 30, all the fish were euthanized and weighed. Weight of the liver, heart, gonads, gastrointestinal tract and eviscerated carcass weight were taken. Carcasses were frozen at -20 °C for carcass analysis. Carcasses were first cut into pieces and then ground twice using Hobard meat grinder (Model E 232) using a 10-mm die followed by twice through a 6 mm die to homogeneous mass. Samples were analyzed for dry matter, organic matter, ash, nitrogen and fat. Nitrogen was determined by the Kjeldahl technique and lipid by soxhlet method (AOAC 1994).

The basal and treatment groups were significantly different in live weight at the beginning of the actual feeding trail due to the variability in live weight gain during acclimatization period. Statistical analyses were performed using general linear model procedure of SAS (1996). Fish weight of each tank on day 0 was considered as the covariate of the final weights of the fish in respective tanks. Differences were considered significant at $p<0.05$.

Table 1. Ingredients and nutrient composition of the basal diet

Ingredient	%
Hoki meal	28.6
Fish meal	24.5
Pollard	19.0
Meat protein	14.1
Skim milk powder	5.4
Wheat flour	5.4
Blood meal	2.2
Betaine anhydrous	0.3
Fish mineral mixture	0.3
Vitamin mixture	0.2
Salbutamol	+/-
Calculated analysis	Level
Moisture %	8.4
Crude protein %	50.2
Crude fibre %	2.5
N free extract %	18.7
Ash %	14.1
Calcium %	3.9
Total Phosphorous %	2.4
Gross energy (Kcal/Kg)	4215

RESULTS AND DISCUSSION

When fish were weighed after four weeks of acclimatization period (day 0 of the feeding trial), the fish assigned to be fed with salbutamol containing diets were significantly heavier ($p<0.05$) (Table 2). It is not clear why a particular group of fish gained higher weight than the other group. Effects of dietary salbutamol on growth performance are summarized in Table 2. In general 20 ppm dietary salbutamol had no effect on growth performance under our experimental conditions. Since, salbutamol is mainly a repartitioning agent, it is not surprising that the growth rate was not affected. Effects of dietary beta agonists on the growth performance are not conclusive. For example, Mustin and Lovell (1993) reported improved growth rates while Webster et al. (1995) observed reduced weight gain in fish fed dietary beta agonists. The overall feed intake as a percentage of initial body weight increased over the 28 day feeding similar to the reported values by other authors (Storebakken and Austreng, 1987; Vandenberg and Moccia, 1998) for similar weight rainbow trout. This indicates that salbutamol has no visible adverse effects on feed intake and health. Throughout the feeding period, salbuamol treated fish had numerically higher feed intake and, in fact intake in third week was significantly higher than the basal diet fed fish (data not shown). Webster *et al.*, (1995) also found no effect on feed intake due to beta agonist L64496. In contrast, Vandenberg and Moccia (1998) found that ractopamine at 10 ppm level reduced the feed intake while at 20 ppm feed intake was increased.

Vandenberg and Moccia (1998) found that ractopamine increased feed conversion efficiency resulting from reduced feed intake and increased weight gain in rainbow trout. In the present experiment, none of the parameters was favorably affected by salbutamol. Since, weight difference between treated and basal diet fed fish was 17 g at the end of the experiment, it is unlikely feed conversion ratio (FCR) would have been different at any stage of the trial. Webster *et al.* (1995) and Mustin and Lovell, (1995) also did not find significant effect of beta agonists on FCR.

Dietary salbutamol increased the carcass recovery by 3.9 % units from 84.6 to 88.5 and the effect was highly significant ($p<0.01$) (Table 3). The increased carcass recovery indicated higher weight of carcass including gills, skin, eyes and head. Contrary to our findings, Mustin and Lovell (1993) observed 1.3% reduction in carcass recovery in ractopamine treated channel catfish.

Effect of salbutamol on carcass composition was also not significant in our experiment (Table 3). Conflicting results are reported in literature regarding the effects of beta agonists on carcass composition of fish. Mustin and Lovell, (1993) and Webster *et al.*, (1995) found that muscle protein and fat contents were reduced in channel catfish given ractopamine and L644, 969, respectively whereas Vandenberg and Moccia (1998) could not find a significant effect due to ractopamine in rainbow trout. However, the experimental conditions such as the agonist used, the fish species and the method of carcass composition determined vary widely among these experiments. Both Mustin and Lovell, (1993; Webster *et al.*, (1995) analyzed the muscle while Vandenberg and Moccia (1998) and the present experiment analyzed the whole carcass. How the carcass composition is determined seems to have a major impact on the results. In salmonids mesenteries, liver, dark muscle and pyloric caeca are the major sites of fat storage (Sheridan, 1994). In whole carcass analysis liver, mesenteries and pyloric caeca are excluded. Therefore, fat contents of these organs or of the muscle would have given a better idea than whole carcass analysis. In general dietary beta agonists have been more effective in channel catfish than in rainbow trout. Fabbri *et al.*, (1995) found that beta adrenergic receptor concentration in hepatic membranes was five times higher in channel catfish than rainbow trout. If this trend exists in other tissues such as adipose tissues and skeletal muscle, low adrenergic receptor concentration in rainbow trout may be the reason for the less effectiveness of beta agonists in rainbow trout. Alternatively differences may be due to different experimental conditions such as drug, dosage, age of the fish and etc.

Though the carcass protein content of the present experiment was similar to values reported elsewhere (Vandenberg and Moccia, 1998), the fat content was low. This may be due to the use of wild strains and the low fat contents of the diets compared to other

Table 2. Effects of salbutamol on body weight gain, feed intake and feed conversion ratio (FCR)

Treatment	day	body weight (g)[1]	carcass weight (g)[2]	weight gain (%)[1]	feed intake % BW	FCR (g/ g)
Control	0	318.6±5	302.9±16.2			
	30	404.4±8	342.3±12	26.9±2.4	1.54±0.3	1.70±0.5
20 ppm salbutamol	0	329.3±5.3	297.9±11.8			
	30	421.6±8.2	373.3±11.5	28±0.5	1.69±0.3	1.72±0.12
leevel of significance	0	*	ns	ns	ns	ns
	30	ns	*	ns	ns	ns

* $p<0.05$, ns not significant,

1 mean of three tanks with 15 fish per tank

2. mean of three tanks with three fish on day 0 and 15 fish on day 30

Table 3. Effects of salbutamol on the carcass recovery and body composition parameters of rainbow trout.

Treatment	day	carcass recovery %	DM %	Protein[1] %	fat[1] %	ash[2] %
Control	0	87.4±1.6	25.38±±0.32	72.3±0.6	17.8±1.5	10.13±0.3
	30	84.6±0.2	27.42±0.38	66.9±1.1	23.9±0.2	8.82±0.07
20 ppm salbutamol	0	86.4±1.4	26.4±0.33	71.4±0.5	19±0.4	9.93±0.07
	30	88.5±0.3	28.16±0.29	66.4±0.9	24.1±0.9	8.96±0.9
leevel of significance	0	ns	ns	ns	ns	ns
	30	**		ns	ns	ns

1. Dry matter basis

2 wet matter basis

** $p<0.01$

ns not significant

Experiments.

The weights of the liver, heart, gonads and total viscera were not affected by the dietary salbutamol (Table 4). Webster *et al.*, (1995) also reported similar results. However, Sota *et al.*, (1995) found that liver, heart and lung weights were increased in lambs given 2 ppm salbutamol. Interestingly, in our experiment 20 ppm salbutamol significantly increased the kidney weight. Sota *et al.*, (1995) also found that salbutamol increased the kidney weight in lambs. Since salbutamol is a selectively beta 2 agonist and vertebrate kidney has a predominance of beta 1 receptors (Timmerman,1987), this observation is surprising and it is unlikely that salbutamol has a direct effect on kidney. Liver and kidney play an important role in the excretion of salbutamol residues (Smith, 1998). On the other hand, many beta agonists have found to increase heart rate and blood flow to many organs (Beerman *et al.*, 1986). Since trout myocardium is exclusively of a beta 2 type organ (Gamperl *et al.*, 1994), it can be assumed that increased kidney weight could be due to increased blood flow to the kidney.

In general, effects of dietary salbutamol at 20 ppm level didn't produce the effects expected. The major limitation of the present experiment was that we tested only two doses (0 and 20 ppm). Therefore, it is too early to species in question, age of the animal, dose of the agonist, duration of the treatment and etc. Therefore, further research, using different doses, age groups of fish and durations are needed to confirm the findings of the present study.

Table 4. Effects of salbutamol on the tissue and organ weights of rainbow trout.

Treatment	day	Liver (g)	Heart (g)	Kidney (g)
Control	0	5.22±1.5	0.4±0.07	2.88±0.22
	30	5.97±1.18	0.58	2.29±0.1
20 ppm salbutamol	0	4.61±0.82	0.41±0.1	3.11±0.31
	30	6.21±1.18	0.57±0.1	3.02±0.13
level of significance	0	ns	ns	ns
	30	ns	ns	**

1. Dry matter basis

2 wet matter basis

** p<0.01

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