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Full Length Research Paper

Effects of different water temperatures and growth agent (recombinant human somatotropin) (r-hGH) on features such as growth, condition factor, feed conversion ratio, survival rate and meat composition of rainbow trout *(Oncorhynchus mykiss)* fingerlings

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In this study, the effects of temperature and different levels of recombinant human somatotropin, one of the growth agents, on growth performances and body composition of the rainbow trout (*Oncorhynchus mykiss*) were investigated. Two different temperatures (10.1 ± 0.10°C and 14.8 ± 0.50°C) and four different doses of the growth agent were added to the feed (0.0, 27.4, 54.8 and 82.2 µg/g) in this experiment. The following results were obtained from the research conducted according to 2 x 4 x 2 randomized factorial experimental design which lasted for a total of 230 days (114 feeding days with growth agent). At the end of the experiment, weights of the fingerlings whose initial weights were 0.23 ± 0.04 g, differed between applications. While the highest value was obtained as 17.51 ± 0.56 g from the 27.4 µg/g growth agent at higher temperature group, the lowest values were obtained as 7.05 ± 0.06 g from the control group at normal temperature and 8.35 ± 0.19 g from the 82.2 µg/g growth agent normal water temperature group. The differences between water temperatures and levels of growth agents were found to be statistically significant ($p < 0.01$). In terms of feed conversion ratio (FCR), different values were obtained from the groups and the differences between them were also found to be statistically significant ($p < 0.01$). While the best feed conversion ratio was obtained as 1.43 ± 0.24 and 1.43 ± 0.23 g from the 27.4 and 54.8 µg/g growth agents at normal temperature groups, the lowest value was obtained as 1.85 ± 0.11 in the control group at higher temperature. From the survival rate (SR) point of view, the difference between the results obtained from the groups was found to be statistically significant in terms of water temperatures, but not significant in terms of the levels of growth agent ($p < 0.05$). As a result of proximate analyses of fish meat, the differences between water temperatures and levels of growth agents were found to be statistically significant in terms of crude protein and crude lipid values ($p < 0.01$). The crude protein ratio was found to be higher and the crude lipid ratio was found to be lower in the groups given the growth agent. After investigating the results obtained from other analyses at the end of the study, the carcass weight was found to be statistically significant ($p < 0.01$), the condition factor (CF) was not found to be significant, and values of hepatosomatic index (HSI) and viscerosomatic index (VSI) were found to be statistically significant ($p < 0.01$) in terms of water temperatures and levels of the growth agent. According to GC-MS (gas chromatography-mass spectrophotometer) tests, residue of the growth agent was found to be negative in the body of fries 15 days after addition to the feed was ceased.

Key words: Water temperature, recombinant human somatotropin hormone (r-hGH), *Oncorhynchus mykiss*, growth performance, meat composition, residue.

INTRODUCTION

The rapid growth of the world population has brought about nutritional problems. The consumers, especially in developed countries, demand cheaper meat with less amount of fat in it. As a result of this high demand of con-

sumers, the use of hormones and other stimulators mostly in sheep, cattle and pig rations has become a current issue. With the use of these compounds, an appreciable increase has been achieved in animal food production, which can be considered a revolution (Oksbjerg et al., 1996; Dunshea et al., 2002).

However, in recent years, there have been some arguments especially in European countries that the products produced by adding this type of anabolic steroids in animal feed have carcinogenic effects. Although these arguments have had negative effects on the studies (Reeds and Mersmann, 1991), the studies on this issue have still going on. These arguments have caused scientists to focus their attention more on growth agents having hormonal effect such as β-agonist and biotechnologically produced somatotropin rather than hormones in meat production. It has been understood that these agents, which are produced artificially and used as injection, implantation and additives to feeds, do not have any negative effects on human and animal health and they have been allowed for use in many countries (Burton et al., 1994; Crome et al., 1996). Particularly, western industrialized countries have been using these agents commonly in order to increase meat and milk production in livestock. The aim is to affect the animal's metabolic system and obtain lower lipid rate but more meat and milk from a unit animal in a shorter time and with less feed (Lawri, 1991; Dunshea et al., 2002).

It has been reported that agonistic agents are also effective in fish as they are in warm-blooded animals, and particularly, growth hormones both increase the appetite in fish and improve the feed efficiency and as a result salmon fingerlings reach the selling weight faster (Mclean et al., 1997; Satpathy et al., 2001; Rønsholdt and McLean, 2004; Gahr et al., 2005; Liu et al., 2007). Water temperature plays an important role in feed efficiency and growth of the trout. Optimal water temperature is very important in terms of both feed efficiency and growth. The rainbow trout generally live between 1 and 20°C, but can resist up to 30°C critical water temperatures. The optimum water temperature is 15°C for them. Since body temperature in fish is not constant unlike warm-blooded animals, the metabolism and therefore the growth is directly dependant on the water temperature (Alanara, 1994; Celikkale, 2002; Pillay and Kutty, 2005).

In this study, we investigated the effects of different levels of recombinant human somatotropin (r-hGH) and different temperatures on growth parameters such as specific growth rate (SGR), feed conversion ratio (FCR), survival rate (SR), condition factor (CF), body composition and residue levels in rainbow trout fingerlings (*Oncorhynchus mykiss)*.

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MATERIALS AND METHODS

In this study, a total of 800 fingerlings which had 0.23 ± 0.04 g average weight were randomly selected among approximately 50,000 fries which were raised in the Trout Fingerling Production and Research Center, Department of Aquaculture, Faculty of Agriculture, Ataturk University. They were used in the experiment by putting 50 fingerlings in each one of 16 tanks. The average normal water temperature during the experiment was 10.1 ± 0.10°C and the heated water temperature was measured to be 14.8 ± 0.50°C. Thermostat heater (10 to 80°C) was used to keep the water temperatures almost constant throughout the experiment.

Commercial extruded trout feed was used as the feed material according to the size of the fingerlings (No: 0-1-2-3). The doses of the growth agent added to the feeds were calculated as (0.0, 27.4, 54.8 and 82.2 μg/g) by taking the results of other studies conducted with similar purposes (McLean et al., 1997; Moccia et al., 1998; Vandenberg et al., 1998) into consideration.

The growth agent somatotropin, which is generally given in the form of injection, implantation and feed additive, is one of the synthetically obtained stimulants which have hormone effects (Burton et al., 1994; Karabulut, 2008). After the related agent doses were weighed on a precision scale (±1 mg) and set at 0.0, 27.4, 54.8 and 82.2 μg/g, they were first dissolved in pure alcohol and then sprayed on the feed (Vandenberg et al., 1998).

The amount of the commercial feeds to be sprayed with the growth agent was calculated from the existing tables which were based on living fish percentage weight and water temperature (Celikkale, 2002; Halver and Hardy, 2002).

At the end of the experiment, 4 fingerlings randomly chosen from each of the 16 units were weighed on a ±1 mg precision digital scale, their weights were determined, their total length was measured with 0.1 cm error, and their values of head, fin, carcass weight, hepatosomatic index and viscerosomatic index were measured respectively in order to determine meat composition as described by Moccia et al. (1998).

Residue analysis in the fish meat was performed in Izmir Bornova Veterinary Control and Research Institute, Department of Toxicology Laboratories by modifying the method stated by Heitzman (1994). According to this method, after the muscle specimen obtained from the fingerlings was processed through various operations, it was placed in GS-MS (gas chromategraphy/mass spectrophotometer) and the results were obtained from a computer. The study was established according to the simple experimental plan of 2 x 4 x 2 (Yildiz and Bircan, 1991), while some of the data obtained at the end of the experiment were analysed according to the “Statistica for Windows”, and others were analysed according to the SAS package program (Duncan, 1971; Hellwig, 1981). Results of the analysis were evaluated based on the formulas below:

Specific growth rate (SGR) (%) = (ln last weight - ln first weight) / growing period (day) x 100 (Fowler, 1991)

Feed conversion ratio (FCR) = $F/(A_2+D- A_1)$ (Celikkale, 2002). Where, F = feed amount of one of the period (g), A_1 = weight at the beginning of period (g), A_2 = weight at the end of period (g) and D = weight of fishes that died in the period (g).

Survival rate (SR) (%) = Fish quantity at the end of period/fish quantity at the beginning of period x 100 (Fowler, 1991).

Condition factor (CF) (%) = (W) weight (g)/(L^3) total length (cm) x 100 (Avsar, 2005).

Hepatosomatic index (HSI) (%) = liver weight (g)/body weight (g) x 100

Table 1. Data obtained from the study.

Group	Normal water temperature				High water temperature			
	Dose of growth agent (µg/g feed)				Dose of growth agent (µg/g feed)			
	0.0 X±Sx	27.4 X±Sx	54.8 X±Sx	82.2 X±Sx	0.0 X±Sx	27.4 X±Sx	54.8 X±Sx	82.2 X±Sx
Initial fish number	100	100	100	100	100	100	100	100
Final fish number	94	98	98	97	100	100	100	100
Mean initial weight (g)	0.23±0.00	0.23±0.00	0.21±0.00	0.24±0.00	0.23±0.00	0.21±0.00	0.22±0.00	0.24±0.00
Mean final weight (g)	7.05±0.06	9.18±0.00	8.77±0.10	8.35±0.19	13.05±0.21	17.51±0.56	16.15±0.12	15.70±0.22
Total feed given per fish (g)	10.56±0.01	13.79±0.01	13.23±0.12	12.61±0.29	23.92±0.29	29.24±1.10	28.78±0.02	28.01±0.49
Mean Weight Increase (g)	6.82±0.06	8.96±0.01	8.56±0.09	8.13±0.18	12.82±0.20	17.29±0.55	15.92±0.13	15.48±0.21
Specific Growth Rate (%)	2.53±0.33	2.74±0.48	2.74±0.51	2.67±0.51	3.00±0.62	3.25±0.80	3.15±0.74	3.16±0.72
Feed Conversion Ratio	1.51±0.19	1.43±0.24	1.43±0.23	1.47±0.24	1.85±0.11	1.68±0.18	1.75±0.16	1.75±0.15
Survival Rate (%)	99.31±1.20	99.77±0.94	99.77±0.65	99.66±1.02	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00

X±Sx = Mean value ± standard deviation of mean value.

Viscerasomatic index (VSI) (%) = inner organ weight (g)/body weight (g) x 100 (Rønsholdt and McLean, 2004).

RESULTS AND DISCUSSION

The results obtained from the study are given in Table 1. The fries, whose average weight was 0.23 ± 0.04 g at the beginning of the experiment reached 7.05 ± 0.06, 9.18 ± 0.00, 8.77 ± 0.10 and 8.35 ± 0.19 g for normal water temperature groups and 13.05 ± 0.21, 17.51 ± 0.56, 16.15 ± 0.12, and 15.70 ± 0.22 g, respectively, for the higher temperature groups at the end of the study. The temperature difference provided a 100% growth increase. As is known, the recommended ideal temperature for trout fingerlings is 8 to 13°C (Alanara, 1994; Celikkale, 2002; Pillay and Kutty, 2005). In addition, it is a known fact in many studies that for each 1°C increase in body temperatures of the trout, their metabolisms also increase as 10% (Alanara, 1994). In this regard, the obtained results seem to confirm our knowledge.

When Table 1 is analysed, the recombinant human somatotropin agent added to the feed was found to have a positive effect on growth. For both temperatures, the best growth results were obtained in 27.4 µg/g dose group. The absolute growth in the normal temperature of 27.4 µg/g group was 2.14 g and was better (31.4% better) than that of the control group. On the other hand, the growth agent (27.4 µg/g) in the higher temperature group provided 4.47 g better absolute weight gain than its control group which was 34.9% higher weight increase. According to the variance analysis, the difference was found to be very significant ($p < 0.01$) between the two temperatures and different growth agent groups.

In a study conducted by Mclean et al. (1997) on Coho (*Oncorhynchus kisutch*) and Chinook (*Oncorhynchus tshawytscha*) salmons, in which they studied 10.0, 30.0 and 100.0 µg/g doses of recombinant bovine growth hormone with injection, they reported that while they could not find a significant difference between treatments until the 8.03 ± 0.75 g fish reached 30 g., higher doses gave better results after they reached 30 g.

A similar result was obtained from a study conducted by Down et al. (1989) on 2.6 ± 0.6 g juvenile Coho salmons (*O. kisutch*). According to this, specific growth rates of 0.2 and 2.0 µg/g levels of recombinant bovine somatotropin after 8 weeks were 2.34 and 2.60, respectively. Their results indicated that the active agent added to the feeds played an important role in the fish growth. The results from our study and theirs indicated that whatever the source of the recombinant growth agent was, it increased the growth when compared to the control group. Another important finding of our study is that the recombinant human somatotropin used in our study gave similar results to the ones which had fish and bovine source.

FCR is the amount of feed given to fish to obtain one unit of increase in fish mass. Steffens and

Table 2. The results obtained from the body composition of the fish.

Group (%)	Normal water temperature				High water temperature			
	Dose of growth agent (µg/g feed)				Dose of growth agent (µg/g feed)			
	0.0 X±Sx (n=4)	27.4 X±Sx (n=4)	54.8 X±Sx (n=4)	82.2 X±Sx (n=4)	0.0 X±Sx (n=4)	27.4 X±Sx (n=4)	54.8 X±Sx (n=4)	82.2 X±Sx (n=4)
Head weight	16.13±0.30	15.88±0.33	13.38±0.67	13.90±0.63	14.62±0.48	14.06±0.06	14.11±0.04	14.21±0.08
Fin weight	2.50±0.03	2.21±0.12	2.25±0.09	2.39±0.01	2.29±0.01	2.23±0.03	2.27±0.01	2.27±0.01
Carcass weight	62.40±0.80	66.14±0.02	64.13±1.53	63.80±0.29	61.13±2.49	67.35±0.49	66.65±0.24	64.28±1.04
Condition factor	1.19±0.01	1.11±0.07	1.15±0.02	1.14±0.02	1.18±0.01	1.09±0.05	1.07±0.02	1.10±0.05
Hepatosomatic index	1.63±0.04	1.41±0.01	1.40±0.11	1.55±0.09	1.25±0.01	1.01±0.01	1.13±0.02	1.21±0.02
Viscerosomatic index	13.77±0.33	12.59±0.90	9.91±1.40	9.49±0.41	12.37±0.50	11.66±0.48	12.41±0.06	12.15±0.06

X±Sx = Mean value ± standard deviation of mean value.

Albract (1974) and Celikkale (2002) reported that feed conversion ratio coefficient should be between 1 and 3. Since more than 50% of the costs of intensive trout breeding consist of feed costs. It is desirable that the feed conversion ratio coefficient is as low as possible. It was reported that this value should not be more than 2 for profitable trout farming (Aras et al., 2000).

The values of FCR coefficient calculated at the end of the study are given in Table 1. According to this, they were obtained as 1.51 ± 0.19, 1.43 ± 0.24, 1.43 ± 0.23 and 1.47 ± 0.24 for the normal temperature groups and 1.85 ± 0.11, 1.68 ± 0.18, 1.75 ± 0.0.16 and 1.75 ± 0.0.15 for the higher temperature groups, respectively. As can be seen from Table 1, the growth agent had a positive effect on feed conversion ratio since FCR was lower for the growth agent groups than the control groups for both temperatures. Another conclusion of these results is that while higher temperature increased growth rate, it gave worse (higher) FCR.

Similarly, Vandenberg et al. (1998) added 5.0, 10.0, 20.0 and 40.0 ppm doses of ractopamine to the trout feed in a 4 week study and observed that the feed conversion ratio of fish fed with 10.0 ppm level feed was lower than the FCR of the control group. The results that we obtained from this study also confirm that the active agent added to the feed improves the feed conversion ratio.

The values of SR obtained during the study are given in Table 1. According to this, the average survival rates (for a period of 14 days) at the end of the study were obtained as 99.31 ± 1.03, 99.77 ± 0.94, 99.77 ± 0.65, 99.66 ± 1.02 for the normal temperature groups and 100.00 ± 0.00, 100.00 ± 0.00, 100.00 ± 0.00 and 100.00 ± 0.00 for the higher temperature groups, respectively. These results indicated that fish deaths were more related to the water temperature than the growth hormone levels. In the intensive trout production, the loss was up to 25 to 30% from fry period to the end of fingerling (Celikkale, 2002). But our SR results were better than that probably, because of the better conditions and better care in the laboratory than in the trout farms.

Although our fish deaths appeared primarily due to temperature difference, there were reports on effect of growth agents on SR in the literature. In fact, in his study about the trout's (*Salmo trutta trutta*) adaptation to sea water, Steffen (1990) injected 2.0 and 8.0 µg/g doses of growth hormone into fish and reported that their survival rate appeared to be higher than those of the control groups. From Table 2, it is observed that the agonistic agent provided better values in the carcass weight, CF, HSI and VSI both in the normal temperature groups and higher temperature groups. The best results were obtained in the 27.4 µg/g dose groups. According to variance analysis, the effect of difference on carcass weight, condition factor, hepatosomatic index and viscerosomatic index was found to be very significant ($p < 0.01$) both between the different temperatures and the different dose groups.

There were similar results confirming our conclusions in the literature. For example, in a study conducted by Vandenberg and Moccia (1998), 0, 5, 10, 20 and 40 ppm of ractopamine was added to the trout feed, and it was concluded that the carcass composition and growth performance were higher in the group with 10 ppm

Table 3. The results of the body proximate and residue analyses of the fish at the end of the study.

Group	Normal water temperature				High water temperature			
	Doses of growth agent (µg/g feed)				Doses of growth agent (µg/g feed)			
	0.0 X±Sx (n=4)	27.4 X±Sx (n=4)	54.8 X±Sx (n=4)	82.2 X±Sx (n=4)	0.0 X±Sx (n=4)	27.4 X±Sx (n=4)	54.8 X±Sx (n=4)	82.2 X±Sx (n=4)
Dry Matter (%)	23.72 ±0.39	24.93±0.08	24.76±0.19	24.53 ±0.02	23.96±0.07	25.15±0.21	24.94±0.07	24.82±0.00
Protein (%)	18.44±0.02	19.05±0.07	18.74±0.02	18.61±0.09	18.88±0.04	19.26±0.09	19.03±0.12	18.88±0.02
lipid (%)	5.17±0.04	3.31±0.03	3.75±0.09	3.97±0.09	4.99±0.09	3.02±0.07	3.47±0.09	3.73±0.20
Water (%)	76.28±0.39	75.07±0.08	75.24±0.19	75.47±0.02	76.04±0.07	74.84±0.21	75.05±0.07	75.17±0.00
Ash (%)	1.39±0.02	1.58±0.00	1.52±0.01	1.49±0.01	1.42±0.02	1.67±0.01	1.61±0.04	1.57±0.02
Ph	6.83±0.01	6.92±0.07	6.91±0.12	6.89±0.12	6.83±0.07	6.93±0.07	6.91±0.02	6.91±0.01
Residue analysis								
1st Day	-	+	+	+	-	+	+	+
15th Day	-	-	-	-	-	-	-	-

(+) With residue; (-) no residue; X±Sx = mean value ± standard deviation of mean value.

level than the control groups after 8 weeks.

Table 2 also shows the results of condition factor. If the condition factor was lower than 0.1, then the condition of the fish was weak. In other words, the fish had long and thin structure. If the condition was equal to 1, the fish had a better condition. However, if the condition factor was higher than 1, the fish was plump (Avsar, 2005). The results in Table 2 indicate that the growth agent groups had better (closer to 1) condition factor than the control groups and the growth agent had positive effect on the condition factor.

The body composition results obtained by analyzing 4 fishes from each tank at the end of the experiment indicated that the agonistic agent increased the carcass protein ratio, but reduced the carcass lipid ratio and thus provided a better meat quality both in the normal temperature and higher temperature groups. The 27.4 mg/g groups gave the best results for both temperatures. According to the results of variance analysis, the effect of the growth agent on the amounts of carcass protein and carcass lipid was found to be statistically significant ($p < 0.01$).

Our result on effects of growth agent on protein and lipid ratios was also confirmed by other studies. In their 12 weeks study, Vandenberg and Moccia (1998) gave 0, 5, 10, 20 and 40 ppm doses of ractopamine to rainbow trout (*O. mykiss)*, and the trouts which were given 10 ppm dose showed a significant increase in the carcass protein ratio, but a significant decrease in the carcass lipid ratio when compared to the control groups. In another study conducted among the rainbow trout (*O. mykiss)* which were fed with 3 different protein levels (25, 35 and 45%) with 0 and 10 $mgkg^{-1}$ ractopamine, it was found that the carcass protein rate was higher, whereas the carcass lipid rate was lower in the fish which were given 10 $mgkg^{-1}$ ractopamine level when compared to the control groups (Moccia et al., 1998).

Some researchers reported that β-agonists increase protein synthesis by retaining nitrogen (Beermann, 2005). Still others reported that β-agonists increase hypertrophy muscle in muscle type II (Gahr et al., 2005; Liu et al., 2007). The findings in all of these studies and our study proved that the growth agent that was added to the feed, whatever source it came from, increased the carcass protein in fish and played an important role in reducing the carcass lipid rate.

We also analyzed the growth agent residue in fish meat in this study. According to Table 3, residues were found in the fish specimens taken on the 1st day when the growth agent was ceased, but no residue of the growth agent was found in the specimens taken on the 15th, 30th and 60th day. This is confirmed by the study of Kaya et al. (1997). They reported that considering

the amount of doses given to animals, minimum of 20 and maximum of 60 days is needed in order to completely discard anabolic substances from the body.

The findings of the study are compatible with the studies conducted on this subject. By adding the growth agents to the fish feeds, fish with low lipid rate and high protein rate were produced with less feed and in shorter time. The residues of the growth agent in the body of the fish disappeared 15 days after ceasing the growth agent.

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