EFFECT OF RECOMBINANT BOVINE GROWTH HORMONE (rbGH) ADMINISTRATION ON GROWTH, BODY COMPOSITION AND GUT PROTEOLYTIC ENZYME ACTIVITY IN FINGERLINGS OF CHANNA PUNCTATUS (BLOCH)

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To study the effect of recombinant bovine growth hormone (rbGH) on growth performance, single intraperitoneal injection (10, 20 and 50 μg/g BW) at the beginning was given to the fingerlings of Channa punctatus maintained under laboratory conditions (LD 12: 12 at 25°C) for 80 days. Variables tested were weight gain, length gain, condition factor, FCR, body composition, VSI, HSI, liver glycogen and proteolytic enzyme activity. Irrespective of the dose of hormone, rbGH treated fish showed high growth performance (SGR, % increase in BW) in comparison with the saline injected control. Treatment of fish with rbGH also improved nutrient retention, feed conversion efficiency and apparent protein digestibility. Viscero-somatic index (VSI) and proteolytic enzyme activity were also high in hormone treated groups. Hepatosomatic index (HSI), liver glycogen and excretion of wastes (N-NH₄⁺ and o-PO₄⁻) were reduced as result of rbGH treatment. Proximate analysis determined at the end of treatment period revealed increased protein and fat content in hormone treated groups as compared with the controls. It is concluded that rbGH is effective in increasing somatic growth in C. punctatus.

INTRODUCTION

In recent years there have been several studies examining the growth promoting abilities of growth hormone (GH) in salmonids (Donaldson et al., 1979; Down et al., 1988, 1989; Bin et al., 2001; Rasmussen et al., 2001). To stimulate growth and improve feed efficiency in finfish aquaculture, Sekine et al. (1985) have reported that intraperitoneal injections of a partially purified preparation of the recombinant hormone in rainbow trout once a week resulted in an increase in growth rate similar to that observed by injecting natural chum salmon growth hormone. Gill et al. (1985) have compared the effect of once a week intraperitoneal injections of recombinant chicken and bovine growth hormones to natural bovine growth hormone in juvenile coho salmon. They found that both the recombinant hormones produced a significant dose dependent increase in growth rate and improved feed conversion in a manner comparable to similar doses of natural bovine growth hormone.
Adelman (1977) administered natural bovine growth hormone to carp by intraperitoneal injection and observed a dose response like stimulation in growth. Condition factors of the treated fish also increased, indicating that fish weight increased more rapidly than fish length. Injected control fish grew at a significantly lower rate than the uninjected controls, apparently due to the stress of handling. Dose-dependent response of exogenous bovine growth hormone was also observed in striped bass (Hunt et al., 2000). Dose-response effect of recombinant growth hormone has been shown to stimulate the growth in channel catfish (Wilson et al., 1988), grass carp (Zhang, 1993), rainbow trout (Garber et al., 1995), coho and chinook salmon (McLean et al., 1997). Several other studies have also suggested that the exogenous growth hormone administration enhances fish growth by stimulating appetite and also by improving feed and protein conversion (Matty, 1986 and Hunt et al., 2000). Markert et al. (1977) proposed that the exogenous growth hormone may stimulate appetite in salmon by a direct action on centers in the hypothalamus that influence feed intake or by inducing a number of metabolic changes that feedback on the hypothalamic centres to affect appetite. These workers have also suggested that exogenous growth hormone may improve feed and/or protein conversion in salmon by one or more of the following possible mechanisms: (1) stimulation of lipid mobilization and oxidation, (2) an action on the rate of protein synthesis and/or breakdown; and (3) stimulation of insulin synthesis and release.

Since feed accounts more than 50 per cent of the operational costs of commercial production of carnivorous fish species like murrels, any improvement of growth rate and/or feed conversion efficiency could potentially decrease the costs of fish production. Therefore, the current study was undertaken to determine the effect of intraperitoneal injection of recombinant bovine growth hormone (rbGH) on growth rate, feed efficiency and body composition in the fingerlings of Channa punctatus. Effect of rbGH was also examined on VSI, HSI, proteolytic enzyme activity and liver glycogen.

MATERIAL AND METHODS

Specimens of C. punctatus (BW 9.08-10.00 g) were obtained from the local fish dealers. Fish were placed in transparent glass aquaria (60×30×30 cm) kept in an air-conditioned laboratory where the temperature was maintained at 25±1°C and lighting schedule at 12 h of light (0800-2000 hrs) alternating with 12 h of darkness (2000-0800 hrs). Fish were acclimated in the laboratory for seven days prior to the initiation of experiment and fed ad libitum on a formulated diet containing 40% protein.

Recombinant bovine growth hormone (rbGH) was obtained from National Hormone and Peptide Program, Harbor-UCLA Medical Centre, California (USA). A single intraperitoneal injection of 10, 20 and 50 µg/g BW were given to the fish at the beginning of the experiment while the control group was injected with solvent (0.6%
saline). Fifteen fish constituted each group. The volume of injection was kept constant
(0.1 ml) and 24 gauge needle was used. Fish were maintained for 80 days and fed @ 3%
body weight in two installments, at 0800 and 1500 hours, on a formulated diet
containing 40% crude protein. Ration size was adjusted at every 15 days intervals after
bulk weighing of the fish. Individual weight and length of fish was recorded at the
beginning and the end of the experiment for the determination of condition factor (k).
Liver and viscera were extirpated for the calculation of hepato-somatic index (HSI) and
viscero-somatic index (VSI). Liver was also processed for the estimation of glycogen
(Dubois et al., 1956). Intestine was dissected out while keeping the fish at 4°C for the
determination of proteolytic enzyme activity (Kunitz, 1947). Fish carcass was processed
for proximate composition following AOAC (1995). Water samples were collected 6-8 hr
post-feeding for the determination of N-NH₄⁺ and o-PO₄⁻ levels from the holding waters
following APHA (1998).

Duncan Multiple Range Test (Duncan, 1955) was used to evaluate the
differences among treatment groups at the 0.05 level of significance. Group means were
compared by Students 't' test (Snedecor and Cochran, 1982).

RESULTS

Growth and feed utilization

Growth performance in terms of weight gain, specific growth rate were
significantly (p < 0.05) high in rbGH injected groups in comparison with the saline
injected control. In general, effect of rbGH was dose-dependent, i.e. live weight gain and
per cent increase in body weight increased with each increase in the dose of rbGH.
Highest values in per cent gain in body weight (87.23%) was observed in the group
injected with rbGH @ 50 µg/g BW. No increase in length values in treated groups was
observed. Condition factor (k) values were also significantly high in the group injected
with rbGH @ 50 µg/g BW as compared with the control. However, no significant
differences in 'k' values were observed between the lowest dose (10 µg) and control
groups (Table 1).

Feed utilization efficiency values were measured in terms of FCR and PER
(Table 1). Treated groups had improved FCR values which progressively and
significantly (p < 0.05) decreased with each increase in the dose of rbGH and thus lowest
values were observed in the group injected with highest dose of rbGH (50 µg/g BW).
On the other hand, PER values increased with each increase in the dose of rbGH. A
similar trend was also observed with respect to GPR, GER and APD values which were
highest at the highest dose of rbGH. Not much variation was observed in food
consumption values in the treated groups as compared with the control, except at the
high dose (50 µg/g) where significantly high (p < 0.05) values were observed (Table 1).
Table 1. Effect of recombinant bovine growth hormone (rbGH) administration (single intraperitoneal injection) on growth, feed efficiency and nutrient retention in *Channa punctatus*

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Saline control</th>
<th>Treatment (μg/ g BW)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>10.0</td>
<td>20.0</td>
<td>50.0</td>
<td></td>
</tr>
<tr>
<td>Initial live weight (g)</td>
<td>9.31</td>
<td>9.17</td>
<td>9.18</td>
<td>9.36</td>
<td></td>
</tr>
<tr>
<td>Final live weight (g)</td>
<td>15.00</td>
<td>15.30</td>
<td>15.62</td>
<td>17.53</td>
<td></td>
</tr>
<tr>
<td>Live weight gain (g)</td>
<td>5.69±0.04</td>
<td>6.13c±0.03</td>
<td>6.46b±0.03</td>
<td>8.16a±0.03</td>
<td></td>
</tr>
<tr>
<td>Growth per cent gain in body weight</td>
<td>61.11b±0.58</td>
<td>66.81c±0.09</td>
<td>70.32b±0.20</td>
<td>87.23a±0.11</td>
<td></td>
</tr>
<tr>
<td>Specific growth rate (SGR %/day)</td>
<td>0.60b±0.004</td>
<td>0.63c±0.001</td>
<td>0.66b±0.001</td>
<td>0.78a±0.000</td>
<td></td>
</tr>
<tr>
<td>Food consumption per day in percentage body weight</td>
<td>1.54b±0.01</td>
<td>1.54b±0.004</td>
<td>1.55b±0.002</td>
<td>1.72a±0.01</td>
<td></td>
</tr>
<tr>
<td>Feed conversion ratio (FCR)</td>
<td>2.67a±0.02</td>
<td>2.46b±0.01</td>
<td>2.40b±0.01</td>
<td>2.28b±0.01</td>
<td></td>
</tr>
<tr>
<td>Protein efficiency ratio (PER)</td>
<td>0.90b±0.06</td>
<td>1.00c±0.05</td>
<td>1.04b±0.005</td>
<td>1.19a±0.01</td>
<td></td>
</tr>
<tr>
<td>Gross protein retention (GPR)</td>
<td>15.50b±0.57</td>
<td>17.52c±0.17</td>
<td>20.80b±0.44</td>
<td>23.92a±0.40</td>
<td></td>
</tr>
<tr>
<td>Gross energy retention (GER)</td>
<td>10.02b±0.21</td>
<td>11.39c±0.11</td>
<td>13.65b±0.02</td>
<td>16.41a±0.15</td>
<td></td>
</tr>
<tr>
<td>Apparent protein digestibility (APD%)</td>
<td>80.22b±0.06</td>
<td>82.79c±0.01</td>
<td>85.62b±0.01</td>
<td>87.19a±0.00</td>
<td></td>
</tr>
<tr>
<td>Initial mean length (cm)</td>
<td>10.00</td>
<td>10.10</td>
<td>10.10</td>
<td>10.10</td>
<td></td>
</tr>
<tr>
<td>Final mean length (cm)</td>
<td>11.61</td>
<td>11.60</td>
<td>11.83</td>
<td>13.80</td>
<td></td>
</tr>
<tr>
<td>Per cent gain in length</td>
<td>16.17a±0.33</td>
<td>15.23a±0.39</td>
<td>13.58b±0.58</td>
<td>13.84b±0.42</td>
<td></td>
</tr>
<tr>
<td>Mean length specific growth rate</td>
<td>0.18a±0.00</td>
<td>0.17a±0.00</td>
<td>0.15b±0.003</td>
<td>0.16b±0.004</td>
<td></td>
</tr>
<tr>
<td>Condition factor (k)</td>
<td>0.96a±0.008</td>
<td>0.98a±0.003</td>
<td>1.03b±0.002</td>
<td>1.14a±0.02</td>
<td></td>
</tr>
<tr>
<td>N-NH₄ (mg/kg BW /day)</td>
<td>60.13a±1.6</td>
<td>39.62b±0.37</td>
<td>36.76a±0.34</td>
<td>16.25c±1.34</td>
<td></td>
</tr>
<tr>
<td>o-PO₄ (mg/kg BW /day)</td>
<td>49.66a±0.06</td>
<td>44.47a±1.62</td>
<td>36.70a±3.36</td>
<td>18.89c±1.71</td>
<td></td>
</tr>
</tbody>
</table>

All values are mean ±SE of mean. Means with the same letter/s in the same row are not significantly (P < 0.05) different. Data were analysed by Duncan's multiple range test.
Table 2. Effect of recombinant bovine growth hormone (rbGH) administration (single intraperitoneal injection) on viscerosomatic index (VSI), hepatosomatic index (HSI) and carcass composition (% wet weight basis) in Channa punctatus

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Initial value</th>
<th>Saline control</th>
<th>Treatment (μg/g BW)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>10.00</td>
<td>20.00</td>
</tr>
<tr>
<td>Viscero-somatic index (VSI %)</td>
<td>2.89±0.01</td>
<td>3.30(^{b})±0.10</td>
<td>3.52(^{a})±0.02</td>
</tr>
<tr>
<td>Hepato-somatic index (HSI %)</td>
<td>0.86±0.01</td>
<td>1.04(^{a})±0.04</td>
<td>1.05(^{a})±0.03</td>
</tr>
<tr>
<td>Carcass composition (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moisture</td>
<td>78.93±0.02</td>
<td>78.25(^{a})±0.06</td>
<td>77.58(^{a})±0.10</td>
</tr>
<tr>
<td>Crude protein</td>
<td>12.40±0.00</td>
<td>13.82(^{b})±0.20</td>
<td>14.36(^{c})±0.03</td>
</tr>
<tr>
<td>Crude fat</td>
<td>2.31±0.01</td>
<td>2.52(^{b})±0.01</td>
<td>2.93(^{b})±0.01</td>
</tr>
<tr>
<td>Ash</td>
<td>3.15±0.005</td>
<td>3.78(^{a})±0.04</td>
<td>3.60(^{a})±0.003</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>0.48±0.003</td>
<td>0.52(^{b})±0.00</td>
<td>0.62(^{c})±0.00</td>
</tr>
</tbody>
</table>

All values are mean ±SE of mean. Means with the same letter/s in the same row are not significantly (P < 0.05) different. Data were analysed by Duncan's multiple range test and Students 't' test.
N-NH₄⁺excretion and o-PO₄⁻production

Fish injected with the highest dose of rbGH excreted significantly \((p<0.05)\) low levels of N-NH₄⁺ and o-PO₄⁻ in the holding water in comparison with the control and also with respect to the fish injected with low doses of rbGH (Table 1).

VSI and HSI

VSI values were significantly \((p<0.05)\) increased in rbGH injected groups in comparison with the saline injected control (Table 2). However, no significant \((p<0.05)\) differences were observed among different rbGH treated groups. A significant decrease in HSI values in comparison to control was observed in fish injected with the higher dose (50 µg) of rbGH.

Proximate carcass composition

Fish injected with 50 µg rbGH had significantly high \((p<0.05)\) accumulation of protein, fat and phosphorus, followed by 20 and 10 µg rbGH injected groups and control. On the other hand carcass moisture and ash contents in rbGH injected group decreased progressively (Table 2).

Hepatic glycogen and intestinal proteolytic enzyme activity

Liver glycogen levels showed no significant differences in groups injected with lower doses of rbGH (10 and 20 µg) and control, while a significant decrease in glycogen levels was observed at the highest dose of rbGH (50 µg) (Fig.1). Proteolytic enzyme activity in terms of specific activity was high in treated groups as compared with the control and the values increased with each increase in the dose of hormone (Fig. 2).

![Fig.1. Effect of recombinant bovine growth hormone (rbGH) administration on liver glycogen levels in Channa punctatus](image1)

![Fig.2. Effect of recombinant bovine growth hormone (rbGH) administration on intestinal proteolytic enzyme activity in Channa punctatus](image2)
DISCUSSION

Effect on growth

Intraperitoneal administration of rbGH resulted in significant increase in body weight, SGR, PER and food consumption in the murrel while a decrease in FCR values took place. Relative to controls, C. punctatus treated with rbGH in the present studies showed a significant increase in growth as indicated by weight and, to a lesser extent, length. These effects were dose dependent. Gill et al. (1985) and Down et al. (1989) have also demonstrated pronounced and rapid enhancement of body weight in juvenile coho salmon given recombinant bovine or chicken GH. Recombinant salmon GH also stimulated the growth of rainbow trout (Salmo gairdneri) and the activity was indistinguishable from that of natural salmon GH (Sekine et al., 1985; Kawauchi et al., 1986; Schulte et al., 1989). Recently, Silverstein et al. (2000) have also reported the stimulatory effect of rbGH on channel catfish. Similar growth stimulatory effects of GH or rbGH were also reported in different fish species (Hunte et al., 2000).

Physiology: Condition factor (k), Viscero-somatic index (VSI), Hepato-somatic index (HSI), liver glycogen and proteolytic enzyme activity

In addition to the effect of rbGH on somatic growth, it has also influenced the condition factor (k), liver metabolism (HSI), liver glycogen levels, proteolytic enzyme activity and body carcass composition. The decrease in HSI observed in the rbGH treated fish is likely to be the result of lipolytic action of growth hormone which has also been reported in several fish species (Sheridan, 1986). Alternatively, the glycogen stored in liver may be converted to glucose through the process of glycogenolysis for providing immediate source of energy, thus sparing protein for growth and accumulation in body.

Agellon et al. (1988) reported an increase in condition factor in rainbow trout after the administration of rtGH. Higgs et al. (1978) also reported that chinook salmon pituitary extracts enhanced condition factors in coho salmon. This effect may have been due to other hormones or releasing factors present in the extract which could have had a stimulatory role on weight increase. The increase in condition factor in C. punctatus is also similar to those already reported by Wilson et al. (1988) in channel catfish, Denzmann et al. (1990) in rainbow trout and Zhang (1993) in grass carp.

The condition factor and body composition data indicated that the increase in body weight was due to an increase in protein and fat deposition and not due to the expected increase in skeletal growth. Decrease in ash contents also supports that an increase in weight was due to the increase in body fat and protein and not due to an increase in skeletal growth.
High VSI and carcass composition data also confirmed the CF data, i.e., there was an increase in fat contents of fish receiving the rbGH when compared with the control. Wilson et al. (1988) also reported an increase in body fat deposition. However, this study also recorded a decrease in body protein contents of the fish injected with rbGH, which may be attributed to the inability of channel catfish to effectively utilize the protein contained in the higher amount of feed consumed by the fish. On the other hand, an increase in protein contents in C. punctatus may be attributed to the efficiency of the fish to utilize protein of plant origin in the feed. Fish normally require a high protein diet as protein is more rapidly converted to energy than glucose. In tilapia, Oreochromis mossambicus bGH stimulates hepatic glycogen depletion and reduced liver glycogen synthetase activity (Leung et al., 1991). Decrease in glycogen synthesis from glucose for hepatic storage may result in increased plasma glucose available to spare the dietary protein, normally metabolized and used as a source of energy. Growth hormone also activates lipoprotein lipase, which catalyzes triglyceride hydrolysis. The energy from lipid oxidation may also spare dietary protein for growth. It is speculated that significant increase in various indices of nutrient efficiency may have also spared protein nitrogen to be directed toward growth in the present study.

Data on intestinal proteolytic enzyme activity also indicate significantly high specific activity in C. punctatus injected with rbGH. Nevertheless, the present study demonstrated that rbGH is effective in increasing growth through nutrient retention (PER, FCR), decreasing the excretion of wastes (N-NH₄⁺ and o-PCy) and also by altering body composition in C. punctatus similar to channel catfish (Wilson et al., 1988), striped bass (Hunt et al., 2000) and rainbow trout (Rasmussen et al., 2001). In conclusion, we report that rbGH stimulates growth in C. punctatus. More research, however, is needed to determine less labour and time intensive methods for use by the producers.

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