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# A STUDY ON THE GROWTH POTENTIAL OF GILTHEAD SEA BREAM HELD IN A COMMERCIAL RECIRCULATION SYSTEM AND TREATED WITH A BY–PRODUCT FROM THE INDUSTRIAL PRODUCTION OF BOVINE GROWTH HORMONE

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## *Summary*

Gilthead sea bream, maintained in a commercial scale recirculation system, were subjected to three injections (0,5 and 10 $\mu g^{-1}$ body weight) with a by–product from the industrial production of recombinant bovine growth hormone (rbGH). Injections were provided at experiment start and at 3 and 6 weeks. Growth performance of animals was evaluated over a period of 8 weeks (n = 171 per treatment). At trial end fish were examined for proximate composition, fillet yield and visceral indices. No differences were recorded in individual growth performance between the three treatment groups ($P > 0.05$). Examination of protein productive value and protein efficiency ratio indicated approximately 20% of dietary protein was incorporated into animals irrespective of treatment. However incorporation of dietary lipid decreased with increasing dose of rbGH. High dose GH decreased liver weight ($P < 0.05$) when compared to control fish, with a concomitant reduction in hepatosomatic index ($P < 0.05$). Fillet weight and yield was higher in animals treated with 10 $\mu g^{-1}$ body weight dose when compared to low dose rbGH injected fish ($P < 0.05$).

*Key words: gilthead sea bream, growth, by–product of bovine growth hormone*

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## *INTRODUCTION*

Intensive aquaculture of gilthead sea bream has developed rapidly over the last half decade, with a more than doubling in production in Mediterranean Europe, from 26500 t in 1995 to almost 60000 t in 2000 (FEAP, 2001). However, the availability of appropriate production sites for cage farming places a severe restriction upon the further expansion of the industry. One method through which the lack of sites can be overcome is with land–based recirculation facilities. Water reuse systems offer many advantages over traditional methods of fish cultivation, including, but not limited by: the ability to control (optimise) rearing conditions; reduction of environmental impact, and protection of stock from external factors such as pollution events and disease outbreaks (Skjolstrup *et al*., 2000). However, a major disadvantage of recirculation systems is the costs associated with construction and maintenance. Increasing efficiency of production must offset these higher costs. Methods of enhancing profitability of recirculation systems include increased stocking densities and by elite feed management and growth control. With respect to the latter, growth factor technologies offer considerable potential.

Contemporary methods for the industrial production of recombinant bovine growth hormone (rbGH) carry with them a significant tonnage of by–product. Although by–product rbGH expresses a high level of activity in mammalian tibia bioassays (80%+ of normal), quality control standards lead to its disposal and subsequent bioremediation (McLean and Byatt, 2000). Clearly, significant commercial gains could be realised if a use was found for the what at present represents and industrial waste product. The objective of the present trial was two–fold: to determine whether increased growth potential existed in gilthead sea bream maintained in a recirculation facility following treatment with rbGH and to establish whether the by–product offered production advantage. The investigation used intraperitioneal injections at 0,5 and 10 μg $g^{-1}$ body weight sea bream every 2 weeks. The study incorporated whole–body and fillet proximate compositional analyses in order to investigate potential changes induced by treatments.

## *MATERIALS AND METHODS*

### *Animals, Husbandry and Treatments*

Ten–month–old gilthead sea bream (*Sparus aurate*, L.) were maintained in 3 indoor glass fibre tanks (2 x 2 x 1.2 m; n = 171 $tank^{-1}$). The experimental system employed recirculated marine water of the following characteristics: salinity 33 ppt, temperature 23 °C, dissolved oxygen range 5.1–7.0 mg $L^{-1}$. Photoperiod was maintained on a 15h light 9h dark cycle. Fish were fed *ad libitum* on 4.5 mm Ecolife 19 pellets (Biomar A/S, Denmark; carbohydrate 12.1%, lipid 24%, ash 7.3%, protein 48.5%) using pendulum feeders. During

all physical manipulations, fish were anaesthetised using ethylene–glycolmonophenylether (1 ml $L^{-1}$; $C_8H_{10}O_2$, Bie & Berntsen A/S, Denmark). Prior to experiment start, 50 fish from each tank were randomly taken and identified with passive integrated transponder tags (Fish Eagle, UK).

Subsequently, each of the 3 tanks was arbitrarily assigned a treatment, with fish being injected with 0.5 or 10 μg rbGH by–product (Monsanto, USA) $g^{-1}$ body weight $3wk^{-1}$ in 0.1 ml 0.8% saline. The rbGH employed in the present study was a by–product from the commercial production of rbGH which, while rejected from industrial use, retained ≥ 80% activity in mouse tibia bioassay.

Initial mean length and weight of PIT tagged fish were as follows: 168.8±8.4 mm and 104.6±14.9 g (control injected), 170.4±7.4 mm and 107.0±14.3 g (5 μg rbGH injected) and 168.6±6.9 mm and 103.1±11.3 g (10 μg rbGH injected). The remaining 121 animals from each tank received the same treatments but were weighed only. All control fish weighed 108.0±14.6 g, 5 μg rbGH 107.8±13.8 g and 20 μg rbGH 105.3±17.7 g. Stocking densities were thus 4.43, 4.39 and 4.25 kg $m^{-3}$ for control, 5, and 10 μg rbGH groups respectively. No significant differences ($P > 0.05$) in weight were recorded between groups.

*Data acquisition and statistical analyses*

All animals were weighed and measured at 0, 3, 6 and 8 wk, after which the trial was terminated. Fish were then sacrificed by anaesthetic overdose and PIT tagged sea bream frozen (–18° C) for further analyses. Animals were examined for fillet yields, hepatosomatic–, visceral–, and carcass indices. Fillet yield (n = 10 per group) was calculated according to the formula:

$$\text{Fillet yield} = \text{fillet weight(g)/body weight(g)} \times 100\%$$

Somatic indices were calculated thus:

$$\text{Somatic Index} = x \text{ weight(g)/body weight(g)} \times 100\%$$

Where x was liver (n = 15), viscera, or carcass weight (n = 10). Proximate compositions were recorded for whole fish and fillet fractions. For each treatment group, 5 whole fish and fillets were examined for in duplicate for moisture and ash and protein, (Kjeldahl N) according to AOAC (1984), lipid was determined using the chloroform extraction method described by Bligh and Dyer (1959).

Feed conversion (FC), productive values and efficiency ratios were calculated using the following formulae:

Feed Conversion (FC) = weight gain (kg)/amount fed (kg)
Productive Protein Value (PPV) = protein gain (g)/protein fed (g) x 100%
Protein Efficiency Ratio (PER) = weight gain (g)/protein fed (g) x 100%

Productive values and efficiency ratios were also generated for dietary lipid. All statistical analyses were performed using SigmaStat software (Jandel Scientific GmbH, Erkrath, Germany) at the $\alpha=0.05$ level of significance. Differences between treatment mean values were examined using one–way ANOVA and significant differences between treatments isolated using Student–Newman–Keul's multiple comparison procedure (SNK–test). Due to the uneven time steps of the growth trial, differences between treatments were compared using paired t–tests. When normality or equal variance of data could not be attained by data transformations, Kruskal–Wallis' one–way ANOVA on ranks was performed. Any potential tank effect as well as handling/treatment stress was assumed to be identical for each group.

## *RESULTS*

The growth performance of PIT tagged fish from each treatment group is summarised in Table 1. No significant differences ($P > 0.05$), in either mean weights or lengths, were recorded between groups throughout the period of observation. Similar responses in terms of weight gain were also observed in untagged animals. Evaluation of condition factor illustrated no differences between treatment groups ($P > 0.05$), with fish returning values in the range of 2.15–2.27. Total biomass weight of fish treated with 10 µg rbGH $g^{-1}$ body weight was, however, significantly ($P < 0.05$) lower than other treatment groups.

*Table 1. Mean (±SD) individual (PIT–tagged) absolute weight and length gains of treatment groups over time. No differences were recorded between groups for weight or length at individual measuring points although significant (P<0.01) increases in group weight and length occurred over time. (n = 50).*

*Tablica 1. Srednja vrijednost (± SD) pojedinačne težine i dužine tretiranih grupa riba kroz vrijeme istraživanja. Razlike između grupa nisu zapažene u težini i dužini riba pri pojedinačnom mjerenju, iako je zapaženo značajno (P < 0,01) povećanje u težini i dužini kroz vremenski period (n = 50).*

| | 0 µg $g^{-1}$ | | 5 µg $g^{-1}$ | | 10 µg $g^{-1}$ | |
|---|---|---|---|---|---|---|
| Week | Length (cm) | Weight (g) | Length (cm) | Weight (g) | Length (cm) | Weight (g) |
| 3 | 6.5 (±2.1) | 17.8 (±4.9) | 7.0 (±2.4) | 18.3 (±5.3) | 6.6 (±2.0) | 16.9 (±5.8) |
| 6 | 14.8 (±3.0) | 36.1 (±6.8) | 15.8 (±3.5) | 38.9 (±9.7) | 16.0 (±3.2) | 37.1 (±9.1) |
| 8 | 19.0 (±3.1) | 44.9 (±8.2) | 19.8 (±4.0) | 47.5 (±12.2) | 19.6 (±3.4) | 47.0 (±11.5) |

*Table 2. Group total biomass throughout the experimental period. Biomass was calculated as the sum of all recorded individual weights and amount of feed ingested per group. Absolute feed conversion is reported as absolute weight gain divided by the amount of ingested feed. Feed conversion (between treatment procedures) is reported as weight gain divided by the amount of ingested feed between treatments procedures.*

*Tablica 2. Ukupna biomasa u grupama riba za vrijeme trajanja pokusa. Biomasa je izračunana kao zbroj svih zabilježenih individualnih težina i količine hrane utrošene po grupi. Konverzija hrane prikazana je kao prirast ribe podijeljen s količinom utrošene hrane. Konverzija hrane (između različitih tretmana) prikazana je kao prirast ribe podijeljen s količinom utrošene hrane između pojedinih tretmana.*

| | | Initial | Wk 3 | Wk 6 | Wk 8 |
|---|---|---|---|---|---|
| Biomass (Kg) | 0 μg $g^{-1}$ | 18.47 | 21.69 | 25.08 | 26.63 |
| | 5 μg $g^{-1}$ | 18.43 | 21.74 | 25.02 | 26.67 |
| | 10 μg $g^{-1}$ | 17.86 | 21.01 | 24.02 | 25.63 |
| Feed Ingested (Kg) | 0 μg $g^{-1}$ | 0 | 3.86 | 8.41 | 12.68 |
| | 5 μg $g^{-1}$ | 0 | 4.14 | 8.73 | 12.92 |
| | 10 μg $g^{-1}$ | 0 | 4.12 | 8.58 | 12.46 |
| Absolute Feed Conversion | 0 μg $g^{-1}$ | – | 1.32 | 1.35 | 1.61 |
| | 5 μg $g^{-1}$ | – | 1.42 | 1.75 | 1.96 |
| | 10 μg $g^{-1}$ | 1.37 | 1.55 | 1.60 | |
| Feed Conversion (between treatment procedures) | 0 μg $g^{-1}$ | – | 1.32 | 1.38 | 5.31 |
| | 5 μg $g^{-1}$ | – | 1.42 | 2.14 | 3.76 |
| | 10 μg $g^{-1}$ | – | 1.37 | 1.73 | 1.90 |

*Table 3. Nutritional indices calculated from the mean amount of incorporated protein/lipid per amount of ingested protein/lipid from feeding (Production Value) and weight gains per amount of nutritional component ingested (Efficiency Radio).*

*Tablica 3. Hranidbeni indeksi izračunani su iz srednje količine odnosa proteina i lipida kroz količinu konzumiranog proteina i lipida iz hrane i prirast težine kroz količinu konzumirane količine hranidbene komponente*

| | | *0 μg $g^{-1}$* | *5 μg $g^{-1}$* | *10 μg $g^{-1}$* |
|---|---|---|---|---|
| Incorporated (Kg) | Protein | 1.19 | 1.06 | 1.05 |
| | Lipid | 1.35 | 1.23 | 0.96 |
| Ingested (Kg) | Protein | 5.46 | 5.57 | 5.38 |
| | Lipid | 3.17 | 3.23 | 3.12 |
| Productive Value (%) | Protein | 22 | 19 | 20 |
| | Lipid | 43 | 38 | 31 |
| Efficiency Ratio (%) | Protein | 136 | 111 | 136 |
| | Lipid | 234 | 192 | 235 |

*Table 4. Summary of physical characteristics of each treatment group and population standard deviations. Yields are presented relative to animal weight. Statistically significant (P<0.05) differences between groups are denoted by different superscripts (N=10).*

*Tablica 4. Ukupni prikaz fizikalnih karakteristika pojedinih tretmanskih grupa i standardne devijacije populacije. Postoci su prikazani s obzirom na težinu životinja. Statistički značajne (P < 0,05) razlike među grupama označene su različitim slovima (N = 10).*

| | Initial | 0 μg $g^{-1}$ | 5 μg $g^{-1}$ | 10 μg $g^{-1}$ | Std. Dev. |
|---|---|---|---|---|---|
| Animal Weight (g) | $105.53^{a}$ | $143.84^{b}$ | $156.63^{b}$ | $148.86^{b}$ | 26.88 |
| Fillet Weight (g) | $58.60^{a}$ | $81.80^{b}$ | $87.13^{b}$ | $86.36^{b}$ | 11.42 |
| Fillet Yield (%) | $55.49^{a}$ | $56.81^{ab}$ | $55.53^{a}$ | $58.12^{b}$ | 2.08 |
| Carcass Weight (g) | $6.65^{a}$ | $8.55^{ab}$ | $10.72^{b}$ | $10.03^{b}$ | 2.27 |
| Carcass Index (CI) (%) | $6.30^{a}$ | $5.92^{a}$ | $6.98^{a}$ | $6.66^{a}$ | 1.41 |
| Liver Weight (g) | $1.51^{a}$ | $2.11^{b}$ | $1.85^{ab}$ | $1.65^{a}$ | 0.49 |
| Hepatosomatic Index (%) | $1.43^{ab}$ | $1.50^{a}$ | $1.18^{bc}$ | $1.10^{c}$ | 0.35 |
| Visceral Weight (g) | $6.72^{a}$ | $8.32^{b}$ | $8.13^{b}$ | $8.26^{b}$ | 1.40 |
| Visceral Index (VSI) (%) | $6.37^{a}$ | $5.80^{ab}$ | $5.19^{b}$ | $5.54^{b}$ | 0.72 |

*Table 5. Proximate composition (%) of whole fish and population standard deviations (Std. Dev.). N=5 per treatment group.*

*Tablica 5. Kemijski sastav (%) cijelih riba i standardna devijacija (Std. Dev.) N = 5 po tretmanu*

| | Dry Matter (%) | Ash (%) | Oil (%) | Protein (%) |
|---|---|---|---|---|
| Initial | $40.06^{a}$ | $4.43^{a}$ | $17.92^{a}$ | $18.56^{a}$ |
| 0μg $g^{-1}$ | $39.49^{a}$ | $3.85^{a}$ | $18.00^{a}$ | $17.\ 84^{bc}$ |
| 5 μg $g^{-1}$ | $39.63^{a}$ | $3.89^{a}$ | $18.40^{a}$ | $18.\ 20^{ac}$ |
| 10 μg $g^{-1}$ | $37.42^{a}$ | $4.01^{a}$ | $16.52^{a}$ | $17.34^{b}$ |
| Std. Dev. | 1.28 | 0,38 | 1.54 | 0.42 |

*Table 6. Results of proximate composition for fillets (n = 5 in duplicate). Significant differences between treatment groups are noted by different superscripts (P < 0.05).*

*Tablica 6. Rezultati kemijskog sastava fileta (n=5 u duplikatu). Značajne razlike među tretiranim grupama zabilježene su različitim slovima (P < 0,05).*

| | Dry Matter (%) | Ash (%) | Oil (%) | Protein (%) |
|---|---|---|---|---|
| Initial | $34.85^{a}$ | $2.06^{a}$ | $14.64^{a}$ | $19.54^{a}$ |
| 0 μg $g^{-1}$ | $36.39^{a}$ | $1.60^{b}$ | $15.79^{a}$ | $19.84^{a}$ |
| 5 μg $g^{-1}$ | $35.97^{a}$ | $1.58^{b}$ | $15.33^{a}$ | $19.\ 85^{a}$ |
| 10 μg $g^{-1}$ | $35.92^{a}$ | $1.69^{b}$ | $15.64^{a}$ | $19.84^{a}$ |
| Std. Dev. | 1.27 | 0.22 | 1.44 | 0.39 |

The amount of feed ingested by each group increased significantly ($P < 0.05$) throughout the period of study, which was mimicked by and increase in absolute feed conversion over time (Table 2). However, no differences ($P > 0.05$) were recorded between groups for absolute feed conversion (Table 2). Evaluation of feed utilisation efficiency was based upon productive value and efficiency ratio for protein (PPV and PER respectively), with each index being calculated according to total biomass and total feed consumed. The nutritional indices of the feed indicated disparities between treatment groups with approximately 20% of dietary protein being incorporated into the experimental animals, irrespective of treatment received. However, incorporation of dietary lipid decreased with increasing amount of rbGH supplied (Table 3).

Examination of fillet weight and yield revealed that fish injected with 10 μ rbGH $g^{-1}$ returned greater yields than 5 μg rbGH $g^{-1}$ ($P < 0.05$) treated sea bream (Table 4) but no difference was seen between control and high dose groups. Carcass weights were greater in GH treated animals when compared to control groups ($P < 0.05$; Table 4), with the former two groups expressing, on average, >20% larger size than the latter. Visceral weight and index did not differ ($P > 0.05$) between treatments. However, hepatosomatic index was significantly smaller ($P < 0.05$) for GH injected fish when compared against control animals ($P < 0.05$; Table 4). Evaluation of proximate composition of whole fish revealed differences ($P < 0.05$) only for dry matter percentage in high dose GH animals. This elevated moisture content was associated with a decline in oil levels (Table 5). Compositional analyses of the fillet illustrated an overall decrease ($P < 0.05$), in percentage terms, for ash content when compared to initial values (Table 6).

## *DISCUSSION*

A wide range of studies has reported growth acceleration in teleosts using various recombinant growth hormones and methods of administration (review: McLean and Devlin, 2000). And, exogenous GH treatment promotes gilthead sea bream growth (Cavari *et al.,* 1993). The apparent lack of effect of GH upon growth in the present study thus remains anomalous, even though GH clearly had a metabolic impact upon animals. In the present study, the effect of handling and injection stress was considered a major cause of poor growth. In addition, the use of salmonid feed and comparatively low stocking densities likely further reduced individual growth potential due to hierarchy formation and possible nutritional deficiencies. Moreover, exogenous GH induces aggressive behaviour in fish (Johnsson and Bjornsson, 1994). The overall rearing environment and treatment effects (stress) of the present investigation might combine to explain the apparent lack of effect of GH upon sea bream growth.

Danzmann *et al.* (1990), reported that GH administration did not have a major influence on growth of rainbow trout (*Oncorhynchus mykiss*) reared at high temperatures (17° C), but did stimulate steroidogenic and metabolic activities. Additionally, they reported decreasing values for HSI in high ration fish (4.6% of body weight/day). In the current study, growth hormone had a significant effect upon HSI, with treated fish returning smaller livers. One of the major impacts of GH is its ability to alter lipid metabolism and it is assumed that the decline in weight and index of the liver was brought about by a reduction in fat storage by this organ. Thus, the lack of effect in the present study could be explained by a similar lack of effect at high temperature. A central issue to the commercial application of GH technologies, as well as to the responsiveness of fish to such treatments, remains the method of hormone delivery employed (McLean *et al.*, 1997). While injection represents a convenient and effective means of hormone application at intervals of 2–4 weeks for experimental studies, injection treatment would be unrealistic in practical settings due to labour costs and the stress imposed by handling (McLean *et al.*, 1999).

Consideration of biomass production illustrates that the 5 μg rbGH $g^{-1}$ body weight group returned slightly reduced growth when compared against the 10 μg $g^{-1}$ and control groups. Since the consumption of feed was identical for all treatment groups, the lower growth of the 5 μg $g^{-1}$ group was reflected in reduced lipid and protein efficiency ratios. Conversely, the productive protein values were identical for all treatment groups, while the productive lipid values decreased with increasing dosage of GH. These observations, taken conjointly with the data derived for liver, thereby indicate a metabolic effect of GH treatment, resulting in proportionately enhanced protein anabolic activity in the 5 μg $g^{-1}$ groups along with increased lipolysis when compared against control sea bream.

Injection of gilthead sea bream with the highest concentration of rbGH produced fish that returned higher fillet yields. The enhanced yields gained with the high GH treated group were associated with lower dry matter content in the whole animal. Yields are known to vary between populations of fish and throughout production cycles: tending to increase with larger framed animals (Bencze Rora *et al.*, 2001) and during winter months (Sinnott, 2001). Moreover, fatty animals generally return reduced fillet yield as a result of trimming loss (Rora *et al.*, 1998). It is possible therefore that the observed increase in yield from the high GH group was brought about by decreased lipid level within the animal. If this were to be the case, and since it is clear that fillet yields have high economic significance to producers and impact fish processing characteristics, then commercial application of growth factor technology would be associated with gains other than those usually attributed to GH (McLean and Devlin, 2000).

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## *Sažetak*

# ISTRAŽIVANJE MOGUĆNOSTI RASTA KOMARČI DRŽANIH U KOMERCIJALNOM RECIRKULACIJSKOM SISTEMU I TRETIRANIH NUSPROIZVODOM INDUSTRIJSKE PROIZVODNJE GOVEĐEG HORMONA RASTA

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Desetomjesečne komarče (n = 171/bazenu) držane su u tri bazena veličine 2 x 2 x 1,2 m s recirkulirajućom morskom vodom čije su karakteristike bile: salinitet 33‰, temperatura 23° C, otopljeni kisik između 5,1 i 7,0 μg $L^{-1}$. Riba je hranjena *ad libitum* s 4,5 mm peletom uz uporabu hranilice s pendulom. Komarče su tretirane nusproizvodom u industrijskoj proizvodnji rekombiniranog goveđeg hormona rasta (rbGH), a primijenjene su tri injekcije, i to 0,5 i 10μg $g^{-1}$ tjelesne težine. Ribe su tretirane na početku pokusa, te nakon tri i šest tjedana. Pokus je trajao osam tjedana. Na kraju pokusa određeni su kemijski sastav riba, postotak fileta, te visceralni indeksi. U pojedinačnom rastu nisu zabilježene razlike u trima tretiranim grupama ($P > 0,05$). Istraživanje vrijednosti produktivnog proteina i količine efikasnosti proteina pokazalo je da je približno 20% proteina bilo inkorporirano u životinje neovisno o tretmanu. No inkorporacija lipida bila je smanjena povećanjem doze hormona rasta (rbGH). Visoka doza hormona rasta smanjila je težinu jetre ($P < 0,05$) u usporedbi s kontrolom s istodobnim smanjenjem hepatosomatskog indeksa ($P < 0,05$). Težina i postotak fileta bili su viši u životinja tretiranih dozom od 10 μg $g^{-1}$ tjelesne težine u usporedbi s niskom dozom rbGH tretiranih riba ($P < 0,05$).

*Ključne riječi: komarča, rast, nusproizvod goveđeg hormona rasta*

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