Journal of Animal and Veterinary Advances 7 (8): 1002-1007, 2008
ISSN: 1680-5593

The Effects of Estradiol Valerate on Body Composition, Carcass, Viscera, Gonado and Hepatosomatic Indexes of Rainbow Trout, *Oncorhynchus mykiss*

[1]Kenan Gullu, [1]Senol Guzel and [2]Kamil Ekici
[1]Department of Fisheries, Faculty of Agricultural, Yuzuncu Yil University, 65080 Van, Turkey
[2]Department of Food Hygiene and Technology, Faculty of Veterinary Medicine,
Yuzuncu Yil University, 65080 Van, Turkey

Abstract: Mono-sexual trout breeding through the use of steroids is derived from the fact that female trout grow faster than males and have meat of better quality and higher carcass efficiency. Treating male trout with estradiol results a phenotypical change of sex, leading to decreased weight of viscera. Some steroid compounds can lead to different results in body development and composition of various kinds of fish. In this study the effects of oral administration of 20 mg of estradiol valerate per kilogram of administered diet were studied in 35-day-old rainbow trout. The protein, fat, ash and moisture contents of fish-meat, carcass and fillets percentages and the hepato-and gonadosomatic indexes were measured as well as the amount of residual estradiol valerate or metabolite that might have remained in fish-meat. Compared to control, there were no changes in moisture, protein, fat and ash contents of fish-meat and in the fillet percentage of the trout. The mass percentage of viscera and the gonado-and hepatosomatic indexes decreased while the carcass percentage increased. No residual estradiol valerate or metabolite was found in the meat of treated trout more than control trout.

Key words: Estrogen, growth, residue, trout, *Oncorhynchus mykiss*

INTRODUCTION

One of the main objectives of commercial fish production is to produce high quality protein at minimum cost. Over the past few years, this goal has been achieved through genetic selection, new feed formulations and new management techniques. Recently, genetic manipulation which improves the body composition and growth of the fish, has gained much attention. Growth-promoting agents have been widely used to improve the growth and meat quality of trout (Vandenberg and Moccia, 1998), including the use of steroids and their synthetic analogues in fish culture (Matty, 1985; Gannam and Lovell, 1991). Throughout the world, fish farming poses some attention-grabbing topics. For example, mono-sexual trout breeding through the use of steroids is derived from the fact that female trout grow faster than males and have meat of better quality and higher carcass efficiency (Ingram, 1988).

Protein accretion represents the net difference between the rates of protein synthesis and degradation. This process is harmonized by complex interactions between various endogenous growth regulators (Bell *et al*., 1998; Breier, 1999; Ronsholdt and McLean, 2004). During an animal's life the mechanisms that mediate protein accretion adjust to meet the changing metabolic demands of the individual. These changes may reflect modifications in target tissue sensitivities, reductions in the synthesis of specific muscle protein (s), or indicate natural decreases in the supply and actions of specific hormones, their binding proteins and receptors (Garlick *et al*., 1998). Disturbances to the growth-regulating hormonal milieu can negatively impact protein status. Conversely, enhanced presence of growth regulators can promote protein accretion and thus influence body composition (Breier, 1999).

Some steroid compounds can lead to different results in body development and composition of various kinds of fish (Yu *et al*., 1979). It was reported that estradiol did not change the fat and protein content in eel, *Anguilla anguilla*, (Degani, 1986) and red sea bream, *Chrysophrys major* (Woo *et al*., 1993). It was also reported that the application of estradiol in salmon did not change the protein and ash while decreasing fat (Yu *et al*., 1979). The hepatosomatic index (HSI) increased by 17β-estradiol in red sea bream (Woo *et al*., 1993). Komen *et al*. (1989) reported that the application of estradiol decreased gonadosomatic index (GSI) value in common carp.

Corresponding Author: Dr. Kenan Gullu, Department of Fishery, Faculty of Agricultural, Yuzuncu Yil University, TR-65080 Van, Turkey

In many species of cultured finfish, females exhibit higher growth rates than males and attain larger sizes. In addition, in some species, males mature before reaching marketable size. Together, this results in a larger dispersion of sizes and an overall reduction in production. Therefore, there is great interest from the private sector to produce all-female stocks (Piferrer, 2001). Single-sex population technology has been used in aquaculture to increase yield by culturing the faster growing sex while preventing unwanted reproduction (Dunham, 1990). Direct or indirect hormonal sex reversal is one of the techniques that have commonly been in used to produce single-sex populations in aquaculture (Hunter and Donaldson, 1983; Piferrer, 2001). Treating male trout with estradiol results a phenotypical change of sex, leading to decreased weight of viscera. As a result, the carcass is increased in the sex-changed fish (Guzel, 2002).

A comparatively recent trend in salmon farming has been the development and application of high-energy diets, the manufacture of which relies upon extrusion and vacuum-coating technologies that permit incorporation of high levels of lipids. Such aqua-feed formulations offer several advantages (Mayer and McLean, 1995), but also lead to increased fat deposition in both the whole body and fillet fractions. While such lipid buildup might be considered beneficial from a weight-gain perspective, increased feed lipid level decreases slaughter yield and, sometimes, causes other impairments of end-product quality including negative impacts to texture, taste and appearance (Alsted, 1991; Regost *et al*., 2001). These negative effects can be reduced through the use of steroids which have been found to decrease fat storage in fish (Yu *et al*., 1979; Miwa and Inui, 1986).

The valerate derivative of estradiol is a natural estrogen that has a longer half-life and is less expensive than 17β-estradiol (Anonymous, 1990). Although, there are studies of Estradiol valerate (E_2V) use in pets (Kalkan and Ocal, 1997), there are no reports of its effects in fish as valerate. It has been reported that the amount of androgen and estrogen in fish tissues as a result of the application of these hormones was the same as seen in wild fish. Therefore, there are no drawbacks to consuming these fish in terms of human health (Goudie *et al*., 1986; Stickney, 1991; Ariman, 2000; Gullu *et al*., 2007). In order to comply with the recommendations of the United Nations Food and Drug Organization and of the Environment Protection Organization on the type and quantity of chemicals used in fish farming (Ostrowski and Garling, 1988) and with restrictions put into practice in Turkey by the Turkish Ministry of Agriculture General Directorate of Protection and Control (Anonymous, 2000), the question whether E_2V remains in fish meat needs to be resolved.

The present study was conducted to determine the effects of E_2V on body composition, carcass, viscera and gonado-and hepatosomatic indexes of rainbow trout and to measure the amounts of residual E_2V in trout meat.

MATERIALS AND METHODS

This study was carried out in Center of Research and Practice (Yuzuncu Yil University, Fisheries Department). It was conducted over a period of 355 days on 800, thirty 5 day old rainbow trout fry. The E_2V, 20 mg kg^{-1} was treated to treatment group's fish for 56 days of totally period. The treatment group's fish were fed no E_2V feed after 56 days, E_2V treatment period, until the end of the experiment, 299 days. At the beginning of the experiments, the average weight of the specimens was 0.32 g. The fish were kept in fiberglass tanks (2.5×0.8×0.7 m (1.4 m^3) filled with aerated well water. The water temperature and dissolved oxygen were recorded throughout the study. The average temperature was 12.53±0.05°C (min. 8.21±0.05°C; max. 17.12±0.12°C) during the study. The amount of dissolved oxygen and pH of the water were 6.2±0.11 mg L^{-1} and 8.23±0.01, respectively. During the E_2V application period, the water temperature and the soluble oxygen amount of water were 10.32±0.30°C and 6.70±0.01 mg L^{-1}, respectively.

In order to conduct duplicate runs of all the experiments, the fish were divided into 4 groups of 200 specimens each; 2 control and 2 experimental (E_2V) groups. This was done so that the method of random coincidence plots (Yildiz and Bircan, 1991) could be used.

Estradiol valerate, $C_{23}H_{32}O_3$, purchased from Schering Medicine and Medical Corporation, Germany (Istanbul, Turkey) was added to the diet of the experimental groups. 20 mg kg^{-1} feed of estradiol is a commonly used dose (Johnstone *et al*., 1978; Hunter and Donaldson, 1983; Goryczko *et al*., 1991; Parks and Parks, 1991). Therefore, it was used 20 mg kg^{-1} feed dose of E_2V in this experiment. The E_2V was dissolved in a few mL of butyl alcohol and then diluted in 400-mL of 95% ethanol for each kg of diet so that it could be sprayed onto the feed while turning in a mixer. The feed used for the controls was sprayed with ethanol only. The feeding material was left to air-dry overnight to eliminate the alcohol and then stored at 4°C for use during the experiments (Ingram, 1988; Gannam and Lovel, 1991).

The fish were fed a commercial feed obtained from Abalioglu Food Production Plant, Denizli, Turkey. The composition of the feed is given in Table 1. The fish were fed 5-6 times per day with granule feed in the fry period and 3 times a day, *ad-libitum*, with pellet feed from the fingerling period to market size. The amounts of daily

Table 1: The chemical composition of feeds used in the experiment

Parameters	Moisture (%)	Crude protein (%)	Crude fat (%)	Crude fiber (%)	Crude ash (%)	Dietary energy (kcal kg^{-1})
Granule feed	9.85	51.73	12.65	1.82	9.20	4000
Pellet feed	10.48	45.29	13.12	2.80	11.22	3800

Estradiol valerate, 20 mg kg^{-1}, was added in the treatment group

consumption feed were recorded for each group. The experimental group received feed treated with E_2V for 56 days. After this period, they were fed a non-treated diet for the remaining 299 days. The fish in the control group were given feed without E_2V and after this period normal feed was given until the end of the experiment.

At the end of the experiment, after 355 days, 8 fish were randomly taken from each group for residue analysis and 25 fish were taken also at random for measurements of the viscera and carcass and to determine fillet efficiency and the hepato-and gonadosomatic indexes.

Residue analysis in fish muscle: Residue analysis was conducted in the fish muscle to determine the presence of residual estradiol valerate after 299 days from application of E_2V, when fish became market-size (355 days old). Eight fish were randomly selected from the control and study groups (duplicate runs), frozen and put in clean Styrofoam boxes (Anonymous, 2000) for transport to the pharmacology laboratory of the Bornova Veterinary Institute for Control and Research, Izmir, Turkey.

The residue was determined by GC-MS, modifying the procedures reported by Heitzman (1994) and Saeed *et al.* (1999). Two sets of samples were prepared by homogenizing muscle from individual subjects separately and from pooled muscles from each group. The following steps were then carried out for the residue analysis.

Enzymatic hydrolysis: The E_2V hormone was first hydrolyzed by mixing 1 g of homogenized muscle with 300 μL β-glucoronidase in pH 9 buffer followed by incubation at 60°C for 2 h. The enzymes in the liquid phase were separated and 5 mL diethyl ether was added onto the hydrolyzed samples and evaporated to dryness at 40°C using a rotary evaporator. This procedure was repeated twice. The residue was dissolved in 100 μL absolute ethanol and 4 mL distilled water. Sep-Pak chromatographic cartridges (Waters GmbH, Eschborn, Germany) were previously conditioned with 5 mL of methanol and then with 5 mL of distilled water. The residue was injected into these cartridges. In the residue the estradiol valerate was retained on the SPE-cartridge under these conditions. After washing the cartridge, the estradiol valerate was eluted with 2 mL of absolute ethanol. The eluate was transferred into a small sealed.

Derivative formation: To obtain the trimethylsilyl derivative (TMS) needed for chromatographic analysis, the ethanol in the hydrolyzed residue was evaporated and treated with 100 μL bis (trimethylsilyl) trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane (TMCS), mixed in a blender and incubated for one hour at 60°C. After incubation, the mixture was evaporated to dryness under a stream of nitrogen at 50°C and the residue was dissolved in 25 μL iso-octane and injected into the GC-MS (Saeed *et al.*, 1999).

GC-MS analysis: For residue analysis, a Hewlett-Packard (HP) model 6890 gas chromatograph (Agillent, Waldbronn, Germany) with a HP5973 Mass Selective Detector was used to identify and quantify the E_2V in 2 μL aliquots of the TMS-derived samples.

Chromatographic conditions: A 25 m, 0.25 ID fused silica capillary column coated with SE-52 was used. The initial temperature was 100°C, the final temperature 280°C was reached in increments of 20°C min^{-1}. Helium was used as carrier gas at a flow rate of 2 mL min^{-1}. The samples were injected at a temperature of 280°C. For detection, the ionization energy was 70 eV, with full scanning from 40-700.

The chemical analyses were carried out according to the Wendee method. The samples were dried in incubator at 105°C for dry matter. Crude protein (CR) was analyzed with Kjeldahl method. It was used Ether extract in Soxhlet instrument for crude fat (CF). And for ash the samples were burned in oven at 550°C (Akkilic and Sürmen, 1979; Bulgurlu and Ergul, 1978; Anonymous, 1995). The hepatosomatic (HSI) and gonadosomatic (GSI) indexes, carcass (C) and fillet (F) were calculated with the formulas suggested by Akyildiz (1992), Halver (1989) and Hepher (1990).

Comparison of 2 groups (control and treatment) for each parameter was performed using the independent t-test by the SPSS software package.

RESULTS AND DISCUSSION

The differences between treatment and control groups in average weight (304.82±11.0 g; 299.08±9.7 g, respectively) were not significant ($p>0.05$) (Table 2). The growth parameters of this study, condition factor, food conversion rate, survival rate etc. were published in 2006 (Guzel *et al.*, 2006). Treatment group's sex ratios were estimated as 97% female and 3% intersexes. The sex ratios were 51% female and 49% male in the control group

Table 2: The average weight and sex ratios (%) of experimental groups

	Weight (g) (mean±SE)	Sex ratios (%)		
		Male	Female	Intersexes
Control	299.08±9.7*	49	51	-
Application group (20 mg E_2V/kg-diet)	304.82±11.0*	-	97	3

*The difference is not significant ($p>0.05$)

Table 3: Influence of experimental diets on body composition of rainbow trout

	Fish dietary hormone concentration	
Parameters (%)	Control group (mean±SE) (0.0 mg E_2V/kg-diet)	Application group (mean±SE) (20.0 mg E_2V/kg-diet)
Moisture	73.30±0.42	73.16±0.15
Crude protein	19.17±0.17	19.35±0.16
Crude fat	4.32±0.01	4.28±0.42
Crude ash	5.12±0.09	5.22±0.04
Viscera*	11.44±3.47	8.15±1.37
Carcass*	83.70±1.17	86.99±1.40
Fillet	68.14±1.25	67.75±1.68
Hepatosomatic index*	0.94±0.11	0.74±0.05
Gonadosomatic index*	8.41±1.28(♂) 0.11± 0.00(♀)	0.12± 0.00**

* The difference is significant ($p<0.05$) between treated with of E_2V and control groups; ** The average of all individuals of treated group with E_2V

(Table 2). The differences between treatment and control groups in crude protein, fat, ash and moisture of the fish meat were not significant ($p>0.05$) (Table 3). However, the differences between viscera and carcass efficiency, HSI and GSI of the groups were significant ($p<0.05$). The fillet efficiency of the groups was insignificant ($p>0.05$) (Table 3). It was observed that the GSI values of the male fish in the control group ranged from 5.62-11.24%. It was found that the GSI values of the female fish in control and application groups were similar.

The application of E_2V to rainbow trout did not change the chemical structure of the fish meat and the fillet efficiency; however, it increased the carcass proportionally but decreased the weight of viscera, GSI and HSI. It was assumed that the differences in the values of carcass and GSI emanate from the fact that male trout achieve sexual maturity earlier than female trout under normal conditions (Ingram, 1988). As stated in the objectives of this study, this is the reason for using female trout. The fact that the high GSI values of the control group males increased the weight of the viscera of the fish and therefore, decreased the carcass proportionally. It constitutes an economic loss in terms for both the producers and the consumers when the weight of the viscera of the fish increases since they are the parts that are not eaten.

It is considered that the application of E_2V decreased the HSI value could occur in such a way to prevent the storage of some food (such as fat) which was stored in the liver or could prevent the development of cells by degenerating liver cells with a pathological effect (Herman and Kincaid, 1988). It was reported that the application of estradiol increased the HSI value in red sea bream (Woo *et al*., 1993) and rainbow trout (Herman and Kincaid, 1988). The increase in HSI value in these studies was explained by the fact that estradiol increased the production of vitellogen via the liver and has a positive effect on carbohydrate, fat and calcium metabolism. The difference between this study and previous work might be assumed to arise from the difference in the effect of E_2V derivative on HSI.

E_2V residue was found the same as of control fish in the application group. The E_2V did not leave extra residue on rainbow trout as in the case of most of the other estradiols. In previous studies it has been reported that the amount of androgen and estrogen in fish tissues as a result of the application of these hormones was the same as seen in wild fish. Therefore, there are no drawbacks to consuming these fish in terms of human health (Goudie *et al*., 1986; Stickney, 1991; Ariman, 2000; Gullu *et al*., 2007). The results of this study show that there is no extra E_2V and its metabolites residue in the fish and support the use of E_2V in trout culture.

It was found as a result of this study that E_2V given in feed at the rate of 20 mg E_2V/kg-diet did not cause a change in the chemical structure of fish meat, protein, lipid and moisture contents. However, E_2V could not eliminate the negative effects, extremely fat fish, of fatty feed which is the product of new technology and used widely nowadays. The application of E_2V increased the carcass proportionally by decreasing the value of gonadosomatic index and provides an economic advantage. The fact that it does not leave residue in fish is counter to the negative publicity concerning such agents and this study has shown that in terms of residue, estradiol applications to fish can be trusted. However, further studies are needed to determine the effects of E_2V on the liver and other organs of the fish.

ACKNOWLEDGMENT

This study was partially funded by The Headship of Scientific Research Projects, Yuzuncu Yil University (Project number: 99 ZF-040).

REFERENCES

Akkilic, M. and S. Surmen, 1979. The laboratory manual of food materials and animal feeding (in Turkish). Ankara University, Veterinary Faculty No: 357, Ankara, Turkey.

Akyildiz, A.R., 1992. Fish foods and technology (in Turkish). Ankara University, Agricultural Faculty, No: 1280, Ankara, Turkey, pp: 192.

Alsted, N.S., 1991. Studies on the reduction of discharges from fish farms by modification of the diet, In: Cowey, C.B. and C.Y. Cho, Eds. Nutritional strategies and aquaculture waste. University Guelph, Ontario, Canada, pp: 77-89.

Anonymous, 1990. Products Prospectus of Sigma Co., pp: 930.

Anonymous, 1995. AOAC, Official Methods of Analysis. 14th Edn. Association of Official Analytical Chemists, Washington, DC., USA.

Anonymous, 2000. The quality control manual of aquatic products (in Turkish). Minister of Agricultural, General Directorate of Preservation and Control, Ankara, Turkey, pp: 229.

Ariman, H., 2000. Effect of the different water temperatures and various levels of growth agent on the growth features and meat composition of rainbow trout, *Oncorhynchus mykiss* fingerlings (in Turkish). (Ph.D Thesis) University of Ataturk, Erzurum, Turkey.

Bell, A.W., D.E. Bauman, D.H. Beermann and R.J. Harrell, 1998. Nutrition, development and efficacy of growth modifiers in livestock species. J. Nut., 128: 360-363.

Breier, B.H., 1999. Regulation of protein and energy metabolism by the somatotropic axis. Domestic Anim. Endocrinol., 17: 209-218.

Bulgurlu, S. and M. Ergul, 1978. The manual of physical, chemical and biological analyze methods of foods (in Turkish). Ege University, Agricultural Faculty, Izmir, Turkey, pp: 176.

Degani, G., 1986. Effect of combined dietary 17 beta-estradiol and 17 alpha-methyl testosterone on growth and body composition of European eels (*Anguilla anguilla*). Aquaculture, 59: 169-175.

Dunham, R.A., 1990. Production and use of mono sex and sterile fishes in aquaculture. Rev. Aqu. Sci., 2: 1-17.

Gannam, A.L. and R.T. Lovell, 1991. Growth and bone development in channel catfish fed 17α-methyltestosterone in production ponds. J. World Aquacul. Soc., 22: 95-100.

Garlick, P.J., M.A. McNurlan, T. Bark, C.H. Lang, and M.C. Gelato, 1998. Hormonal regulation of protein metabolism in relation to nutrition and disease. J. Nut., 128: 356-359.

Goryczko, K., K. Bieniarz, S. Dobosz and J. Grudniewska, 1991. The effect of 17 β-estradiol on Rainbow trout, *Oncorhynchus mykiss*. Polskie Archiwum Hydrobiology, 38 (2): 303-309.

Goudie, C.A., W.L. Shelton and N.C. Parker, 1986. Tissue distribution and elimination of radiolabeled, methyl testosterone fed to sexually undifferentiated blue tilapia. Aquaculture, 58: 153-162.

Gullu K., S. Guzel, K. Ekici and H.A. Duyar, 2007. Residue analysis and effects of 17α-methyltestosterone on body composition of rainbow trout, *Oncorhynchus mykiss*. Ital. J. Food Sci., 19 (3): 357-362.

Guzel, S., O. Ozden and K. Gullu, 2006. The effect of estradiol valerate administration on growth of rainbow trout, *Oncorhynchus mykiss*. Res. J. Anim. Vet. Sci., 1 (1): 59-61.

Guzel, S., 2002. The effects of estradiol valerate on sex reversal and growth of rainbow trout, *Oncorhynchus mykiss* (in Turkish). (Ph.D Thesis) University of Ege, Izmir, Turkey.

Halver, J.E., 1989. Fish Nutrition. Academic Press, New York, London, pp: 785.

Heitzman, R.J., 1994. Veterinary drug residues. Residues in food Producing animals and their products: Reference materials and method, Published on behalf of commission of the European Communities, Luxembourg.

Hepher, B., 1990. Nutrition of Pond Fishes. Cambridge University Press, Cambridge, New York, pp: 386.

Herman, R.L. and H.L. Kincaid, 1988. Pathological effects of orally administered estradiol to rainbow trout. Aquaculture, 72: 165-172.

Hunter, G.A. and E.M. Donaldson, 1983. Hormonal sex control and its application to fish culture. In: Hoar W.S., D.J. Randall and E.M. Donaldson (Eds.). Fish Physiology, Vol. 9, Part B. Academic Press, New York, pp: 223-291.

Ingram, M., 1988. Farming rainbow trout in fresh water tanks and ponds. In: Laird, L. and T. Needham (Eds.). Salmon and Trout Farming. Ellis Harwood Limited, England, pp: 155-189.

Johnstone, R., T.H. Simpson and A.F. Youngson, 1978. Sex Reversal in Salmonid Culture. Aquaculture, 13: 115-134.

Kalkan, C. and H. Ocal, 1997. Effect of External Estradiol Valerate or Diethylstilbestrol on Luteolysis During the Luteal Period in Cows. Turk. J. Vet. Anim. Sci., 21: 9-11.

Komen, J., P.A.J. Lodder, F. Huskens, C.J.J. Richter and E.A. Huisman, 1989. Effects of oral administration of 17-alpha-methyltestosterone and 17-beta-estradiol on gonadal development in common carp, *Cyprinus carpio*. Aquaculture, 78: 349-363.

Mayer, I. and McLean E., 1995. Biotechnological and bioengineering strategies for reduced waste aquaculture. Water Sci. Technol., 31: 85-102.

Matty, A.J., 1985. Fish Endocrinology. Timber Press Ltd. Portland, USA, pp: 267.

Miwa, S. and Y. Inui, 1986. Inhibitory effects of 17α-methyltestosterone and 17β-estradiol on smoltification of sterilized amago salmon, *Oncorhynchus rhodurus*. Aquaculture, 53: 21-39.

Ostrowski, A.C. and D.L. Garling, 1988. Influences of anabolic hormone treatment and dietary protein; energy ratio on condition and muscle deposition of rainbow trout. The Progressive Fish-Culturist, 50: 136-140.

Parks, L.M. and J.W. Parks, 1991. Sterilization and Feminization of Brook Trout, *Salvelinus fontinalis* by Androgen and Estrogen Treatment. Bulletin of The Aquaculture Association of Canada, 91: 34-35.

Piferrer, F., 2001. Endocrine sex control strategies for feminization of teleost fish. Aquaculture, 197 (1): 229- 281 (53).

Regost, C., J. Arzel, M. Cardinal, M. Laroche and S.J. Kaushik, 2001. Fat deposition and flesh quality in sea water reared, triploid brawn trout, *Salmo trutta* as affected by dietary fat levels and starvation. Aquaculture, 193: 325-345.

Ronsholdt, B. and E. McLean, 2004. Effect of growth hormone and salbutamol on growth performance, filet proximate composition and pigmentation of rainbow trout, *Oncorhynchus mykiss*. Aquaculture, 229: 225-238.

Saeed, T., I. Naimi, N. Ahmad and W.N. Sawaya, 1999. Assessment of the levels of anabolic compounds in Kuwait meat industry: Optimization of a multiresidue method and the results of a preliminary survey. Food Control, 10: 169-174.

Stickney, R.R., 1991. Culture of Salmonid fishes. CRC Press, Florida, pp: 189.

Vandenberg, G.W. and R.D. Moccia, 1998. Growth performance and carcass composition of rainbow trout, *Oncorhynchus mykiss*, fed the beta-agonist ractopamine. Aquacul. Res., 29: 469-479.

Woo, N.Y.S., A.S.B. Chung and T.B. Ng, 1993. Influence of oral administration of estradiol-17 beta and testosterone on growth, digestion, food conversion and metabolism in the under yearling red sea bream, *C. major*. Fish Physiol. Biochem., 10: 377-387.

Yildiz, N. and H. Bircan, 1991. The methods of Research and Experiment (in Turkish). Ataturk University, Agricultural Faculty Pres, Erzurum, Turkey, pp: 227.

Yu, T.C., R.O. Sinnhuber and J.D. Hendricks, 1979. Effect of steroid hormones on the growth of juvenile Coho salmon, *Oncorhynchus kisutch*. Aquaculture, 16: 351-359.