International Council for the Exploration of the Sea

CM 1974/M:20
Anadromous and Catadromous Committee
Ref. E(Fish.Imp. C.)

SOME OBSERVATIONS ON THE USE OF ANABOLIC STEROIDS IN THE CULTURE OF SALMONID FISH

by

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Summary

It has been shown that the steroid ethylestrenol is an effective anabolic substance of potential value in the farming of salmon (S. salar) and rainbow trout (S. gairdneri). The increases in growth rate and food conversion efficiency do not appear to be achieved at the expense of muscle quality. The rate at which ingested ethylestrenol is metabolized and eliminated is consistent with its effectiveness as an oral anabolic and high enough to ensure that residues do not remain in body tissues. It has been established that the chemical stability of ethylestrenol incorporated into fish food is such as to allow its convenient use on a commercial scale.

Introduction

It is clear that major savings in the costs of farming fish for human consumption would accrue if the efficiency of food conversion could be improved and if faster growth rates could be achieved. In animal husbandry anabolic substances, for example methyltestosterone and diethylstilbestrol (Clegg and Cole, 1954) are used to this end. Both substances are however potent sex hormones and may induce undesirable side effects including water retention (Bulkley, 1972) and liver damage (Kruskemper, 1968) in the treated animal. The common practice of attempting to reduce the androgenic effects of methyltestosterone by adding a counterbalancing amount of the estrogenic diethylstilbestrol (Bidner, Merkel, Miller, Ullney and Hoefer, 1972) appeared to us to be less than satisfactory with the real need being for a less harmful anabolic. Of those substances commercially available, ethylestrenol under the trade name Orabolin (Organon Laboratories Ltd) appeared to hold most promise for use in fish farming. This paper describes preliminary experiments in a projected series aimed at assessing its potential value. The inclusion in these experiments of groups of fish treated with methyltestosterone allowed a comparison to be made of the effectiveness of ethylestrenol relative to this steroid.

Methods

Rainbow Trout

240 farm-reared rainbow trout were equally distributed between 4 similar outdoor tanks each supplied with running water at a flow rate of 1.5 litre/minute. Fish were tagged so that individual as well as group weight gains could be monitored. A commercial fish food was used throughout the experiment. Steroid

was incorporated into food which had first been defatted by extraction with ethanol. An ethanolic solution of an appropriate weight of steroid was added and the ethanol removed using a rotary evaporator in vacuo and then by allowing the food to air dry on shallow trays. Treated food was then mixed with an equal weight of normal food to give final steroid concentrations of 2.5 and 12.5 mg of ethylestrenol and 2.5 mg of methyltestosterone per kilogram of food. The control group was fed a 1:1 mixture of normal and defatted food. The four groups were fed with weighed rations calculated from the weight of fish in the group and were watched carefully during feeding to ensure that all the food provided was taken. Fish were weighed and measured at suitable intervals and rations adjusted according to the newly determined weights. After approximately 3 months, 15 fish chosen at random from each group were killed and the livers, gonads and viscera (comprising the complete digestive tract and spleen) were excised and separately weighed. Gonads were fixed in Bouins reagent, sectioned from paraffin and stained with Harris haematoxylin and eosin. Representative samples of each carcass were individually analysed for total nitrogen (microkjeldahl procedure), lipid (Folch extraction) and dry weight by heating at 100°C to constant weight. Visceral lipid was also determined.

Salmon Parr

240 salmon parr from the Laboratory's Smolt Rearing Station at Almondbank Perthshire were distributed into equal groups in 6 similar tanks held indoors and which through shortage of space were arranged in two tiers of three. To counterbalance the effects of any resulting tank to tank variation the groups of fish were rotated between tanks after each weighing. Fish were weighed and measured at approximately monthly intervals over six months by which time the groups had been rotated through all six tanks. The fish were exposed to a 12 hour light - 12 hour dark cycle and were fed from automatic feeders every 20 minutes ad libitum during the light portion of the cycle only. A commercial salmon food was used throughout the experiment. To dispense with a defatting stage prior to steroid incorporation, treated food was stored in the dark at 6°C in an atmosphere of nitrogen and freshly prepared every 10 days. Steroids were incorporated into food using ethanol as previously described to give dose levels of 0.5 mg 2.5 mg and 12.5 mg of ethylestrenol and 2.5 mg and 12.5 mg of methyltestosterone per kilogram of food. The control group received food which had been treated with ethanol only. On termination of the experiment 20 fish were randomly selected from each tank, dissected as previously described and the organs weighed. Gonads were examined histologically. Nitrogen, fat and dry weight values remain to be determined.

The stability of ethylestrenol incorporated into fish food

A mixture of 20 uCi (3.2 ug) of 20, 21-^{3}H(u) ethylestrenol and 0.5 mg of unlabelled ethylestrenol was incorporated into the commercial salmon food with ethanol and the food then enclosed in a transparent-polythene bag and left in the laboratory. At suitable intervals 0.5 g samples of food were removed, extracted with ethanol and the undegraded ethylestrenol separated from the extract and purified by thin layer chromatography and estimated by liquid scintillation counting.

Elimination by rainbow trout of ingested ethylestrenol

A single rainbow trout (ca 180 g) was fed for 2 days on a commercial diet containing 5 mg/Kg of ethylestrenol. It then received a single pellet of food into which had been incorporated 20 uCi of tritiated ethylestrenol. Blood samples were withdrawn after suitable time intervals, and the radioactivity in weighed samples of plasma determined by liquid scintillation counting.

Results and Discussion

The initial lack of radiotracer labelled ethylestrenol precluded a preliminary investigation of the chemical stability of this steroid incorporated into fish food. Although the pure substance shows marked stability, it was anticipated that its rate of oxidative breakdown might be considerably increased when brought into contact with the autoxidizing lipid present in fish food. To lessen the risk of destruction, steroid was incorporated into small batches of salmon food at frequent intervals as required and the treated food then stored under nitrogen. The larger quantities of food required for the trout experiment presented a more serious problem. The most practicable approach appeared to be to defat a portion of the food prior to incorporation of steroid. Food so treated was then cold stored and mixed with an equal quantity of normal food as required. Fish in the control group received food which, except for the exclusion of steroid had been subject to a strictly parallel procedure.

A sample of tritiated ethylestrenol which later became available made possible a preliminary study of the stability of this steroid incorporated into fish food. At least 85% of the material incorporated remained undegraded after storage for one month under conditions which were far removed from ideal. It is now clear that the precautions taken to defat food and store treated food under nitrogen are not necessary and have been discontinued in the experiments currently in progress.

The weight data accumulated over a 3 month period from weighings of individual rainbow trout in the 4 groups are presented graphically in Fig. 1 in which the means of individual percentage increases are plotted against time. It is clear that fish in the 3 groups which received anabolic steroids showed greater increases than did those in the control group ($p < 0.01$). Overall values for the 3 month period are shown in Table 1. The efficiencies with which fish in the 4 groups converted food are also shown and clearly demonstrate the value of added steroid in improving food utilization efficiency. The mean values of carcass nitrogen, dry weight and lipid are also shown. Values for treated groups are not significantly different from those of the control and provide at least a preliminary indication that the increased weight gains achieved with the anabolics are not at the expense of muscle quality. Visceral fat values (see Table 1) of fish in the treated groups are lower ($p < 0.001$) than those of the controls and exemplify the effect of anabolic steroids in promoting muscle synthesis at the expense of depot lipid. These lower lipid values, reflected also in the lower visceral weights (Table 1) may also be indicative of an increased food requirement not fully met by the diet received so that further improvements in weight gain of the treated groups might accrue from increasing the ration further.

There was no significant difference in the ratio of liver to body weights between groups and although histological examination of livers remains to be carried out it would appear that under the conditions of these experiments the anabolics had not induced any noticeable degree of liver damage.

The overall weight gains expressed as percentage increases, of the six groups of salmon parr are shown in Table 2. Statistical analysis of data from the monthly weighings established that the weight increments of the three groups receiving ethylestrenol were significantly higher ($p < 0.01$) than that of the control group but were not significantly different from each other. The average monthly growth increment for the ethylestrenol treated groups was 27% higher than that of the control. Analysis of data from the groups receiving methyltestosterone revealed no significant difference between them nor was their average increment different from that of the control. Visceral weights for all groups are presented in Table 2. Values for groups receiving the 2 higher dose levels of ethylestrenol and the higher dose level of methyltestosterone were significantly lower than that of the control group. Liver weights, as in the trout, were not significantly different from the control.

The gonadosomatic indices of male trout in all the steroid treated groups and of male salmon in all but the group receiving 2.5 mg/Kg of ethylestrenol were significantly higher ($p < 0.05$) than those of controls (see Tables). Histological examination revealed some advancement in the development of testes from all the steroid treated groups. Testes from ethylestrenol treated fish showed hypertrophy of the sperm ducts, the extent of which appeared to be related to the level of steroid received. Testes from fish receiving methyltestosterone were however further advanced to the spermatid stage. The gonadosomatic indices of ovaries from treated fish were not significantly different from controls; ovaries appeared normal by histological examination.

Ethylestrenol has been used as a therapeutic agent in medical and veterinary practice for some years. Its particular advantage over methyltestosterone and diethylstilbestrol lies in its very low androgenic to anabolic index giving this steroid a wide margin of safety in long term use. It is now clear that ethylestrenol is an effective anabolic agent in salmon and rainbow trout which to date has produced no deleterious side effects. Its slight effect on the male gonad is not regarded as important but is under further investigation. Experiments currently in progress with tritiated ethylestrenol suggest that its metabolic half-life (as measured by changes in plasma level with time) is consistent with its activity as an effective anabolic but which nevertheless is quickly and safely eliminated by metabolism and excretion.

Acknowledgements

The authors are grateful to Organon Laboratories Ltd for providing the ethylestrenol used in this work and for the sample of tritiated steroid used in the stability and excretion studies. The assistance of Miss A Davis and Mr G Cooke in this work is acknowledged.

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Table 1

	Total Mean % Inc.Wt	Food Utilization Efficiency	Carcass Analysis (% Carcass Wet Weight)			Viscera (% Total Body Weight)		G.S.I. ♂
			Nitrogen	Dry Wt	Lipid	Lipid	Weight	
Control	97.9	0.40	2.72	24.1	4.44	1.47	13.09	0.048
2.5 mg Ethylestrenol/Kg food	113.3	0.43	2.59	24.2	4.63	0.97	11.31	0.088
2.5 mg Methyltestosterone/Kg food	116.5	0.42	2.62	24.7	5.01	0.92	11.71	0.101
12.5 mg Ethylestrenol/Kg food	122.4	0.47	2.63	25.3	4.78	0.70	10.15	0.085

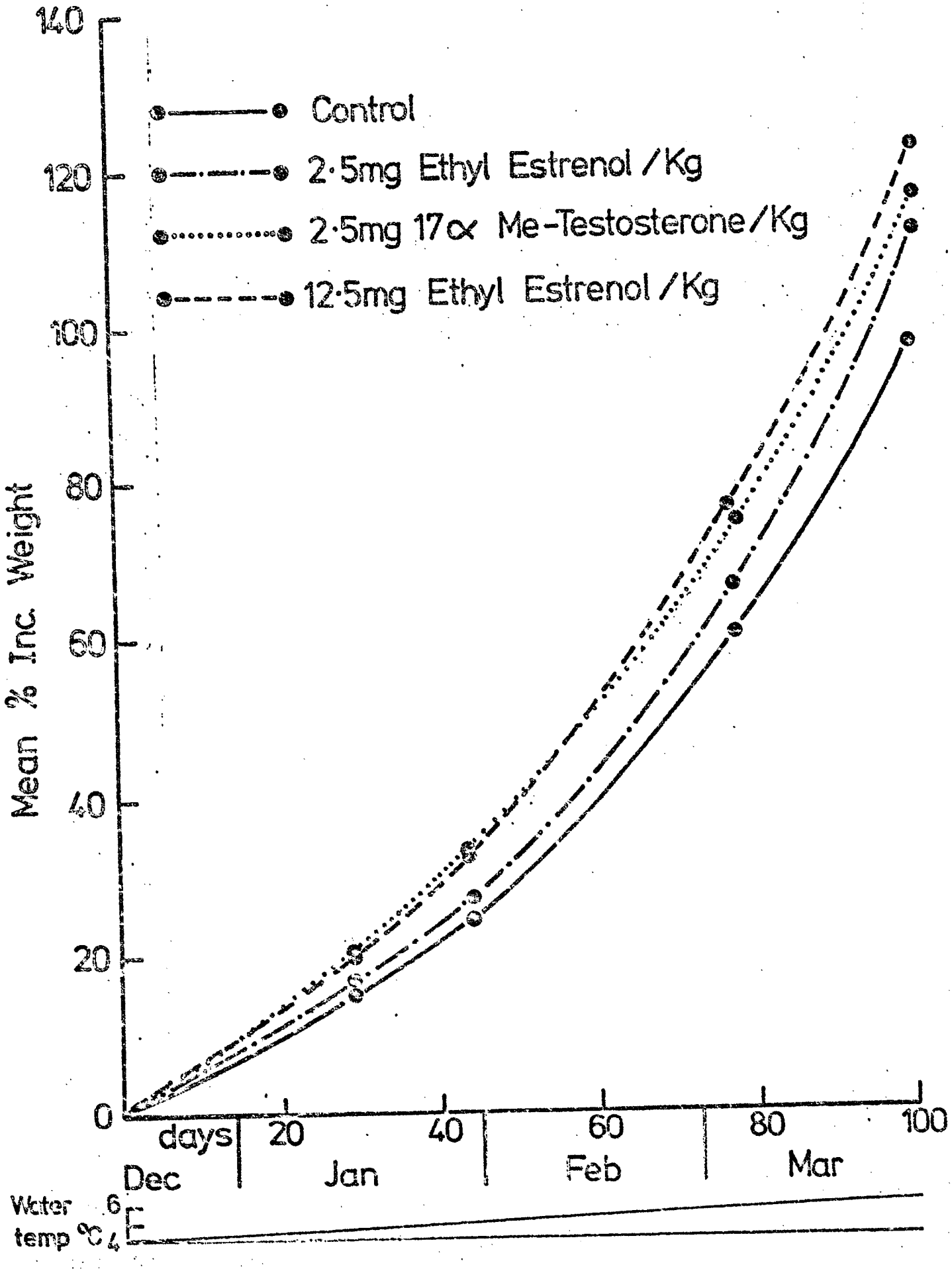
Food Utilization Efficiency - Weight Gain Per Unit Food
G.S.I. - Gonadosomatic Index

Table 2

	Total % Increase Mean Weights	Visceral Weight (% Total Body Weight)	G.S.I. ♂
Control	55.2	10.12	0.064
0.5 mg Ethylestrenol/Kg food	66.7	9.95	0.103
2.5 mg Ethylestrenol/Kg food	67.3	8.64	0.067
12.5 mg Ethylestrenol/Kg food	62.7	8.73	0.099
2.5 mg Methyltestosterone/Kg food	50.5	9.64	0.111
12.5 mg Methyltestosterone/Kg food	47.4	8.53	0.101

G.S.I. - Gonadosomatic Index

Figure 1


140
120
100
80
60
40
20
0
Mean % Inc. Weight
Control
2·5mg Ethyl Estrenol / Kg
2·5mg 17α Me-Testosterone / Kg
12·5mg Ethyl Estrenol / Kg
days
20
40
60
80
100
Dec
Jan
Feb
Mar
Water temp °C
6
4