Effect of dietary protein level, and an anabolic steroid, ethylestrenol, on the growth, food conversion efficiency and protein efficiency ratio of rainbow trout (*Salmo gairdneri*)

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1. Three isoenergetic test diets containing 320, 430 and 530 g protein/kg, with (experimental) and without (control) inclusion of an anabolic steroid, ethylestrenol, were given to rainbow trout (*Salmo gairdneri*) of mean initial weight 27 g, for 60 d.

2. After 60 d, all trout groups were given an identical, steroid-free, commercial diet (410 g protein/kg) for a further 30 d, to observe withdrawal effects on growth.

3. The weight and length of trout given the 430 and 530 g protein/kg control diets were significantly greater after 60 d than those given the 320 g protein/kg control diet.

4. Inclusion of steroid enhanced the weight and length of trout given the 320 and 430 g protein/kg experimental diets, exerting a preferential effect on weight as opposed to length.

5. After steroid withdrawal, a significant difference between the weight and length of the 320 g protein/kg control and experimental groups was still apparent.

6. An increase in dietary protein increased the food conversion efficiency, and decreased the protein efficiency ratio. Inclusion of steroid increased both these factors over the respective controls.

7. Protein assimilation decreased, and faecal nitrogen content increased with increasing dietary protein. In trout given steroid, protein assimilation and apparent digestibility was higher, and facecal N content, lower than the controls.

8. Muscle protein increased with increasing dietary protein, and was higher in trout given steroid.

9. Relative liver weight increased with increasing dietary protein. Inclusion of steroid resulted in a reduction in relative liver and gut weight, and an increase in kidney weight.

10. It is concluded that ethylestrenol promotes both the growth and efficiency of nutrient utilization of trout. The magnitude and duration of these effects are a function of the dietary protein level.

Although a considerable amount of information is now available on the nutritional requirements of fish (Halver, 1972; Cowey, 1975; Cowey & Sargent, 1972, 1977, 1979), studies on the hormonal control of growth are fewer by comparison (Donaldson *et al.* 1979). In all these studies, a diet of fixed nutrient composition has been employed.

It is well established that increasing the protein content of trout diets to approximately 500 g/kg results in improved growth and food conversion rates (Satia, 1974; Austreng, 1976; Pieper & Pfeffer, 1980; Bromley & Smart, 1981). However, this effect also depends on the relative proportions of the other dietary constituents (Castell *et al.* 1972; Dupree *et al.* 1979; Reinitz & Hitzel, 1980; Reinitz & Yu, 1981). Since anabolic steroids increase growth in fish, and that growth is a function of dietary protein content, the possibility was considered that inclusion of a steroid in diets of differing protein levels might exert differential effects on various growth indices. In the present study, three isoenergetic test diets containing 320, 430 and 530 g protein/kg, with or without steroid, were given to trout for 60 d, after which time, all fish were given an identical steroid-free, commercial diet for a further 30 d to observe withdrawal effects on growth. Ethylestrenol, an anabolic-androgenic steroid, was employed, since previous studies have shown this steroid to promote growth in trout and Atlantic salmon (*Salmo salar*) (Simpson, 1976), and in carp (*Cyprinus carpio*) (K. P. Lone, unpublished results). The results showed that ethylestrenol enhances the growth of trout, and also increases the food conversion efficiency (FCE) and

protein efficiency ratio (PER). The magnitude and duration of steroid effects on growth indices, however, appeared to be related to the dietary protein level.

MATERIALS AND METHODS

Experimental conditions

Rainbow trout (*Salmo gairdneri*) were obtained from Burwarton Fish Farm, Cleobury North, Shropshire. Before the experiments, all fish were placed in quarantine for 3 weeks, after which time, thirty-five fish were randomly transferred to each of six 100 l polystyrene tanks, plumbed with a recirculating system, which included gravel filters and faecal traps. Water was delivered at a rate of 120 l per tank/h, and the temperature maintained at $12{\cdot}0 \pm 0{\cdot}2°$. The photoperiod was set on a 12 h light–dark cycle, using fluorescent tubes as the light source. Water ammonia, pH, and oxygen saturation were monitored every 2 d, water temperature once daily (12.00 hours), and the entire system cleaned thrice weekly. During quarantine, and subsequent acclimation to the tanks for 15 d, trout were given a commercial diet (Omega no. 4; Edward Baker Ltd, Sudbury, Suffolk) once per d to satiation, of composition (g/kg): oil 80; protein 470; fibre 45; ash 100; moisture 80; carbohydrate (nitrogen-free extract) 225, (manufacturers analysis).

After acclimation, trout of average weight 26–28 g (range: 21·05–35·84 g), were redistributed so that the mean weight of fish (thirty-five/tank) in the different tanks, and their variances, did not differ significantly.

Diet preparation and feeding regimen

Three isoenergetic test diets, calculated to contain 350, 450 and 550 g protein/kg, were prepared. The composition of the diets, and results of the proximate analyses (according to the Association of Official Analytical Chemists, 1975), are shown in Table 1. The

Table 1. *Composition and proximate analysis of test diets given to rainbow trout* (Salmo gairdneri)*

Component (g/kg diet)	Protein level (g/kg diet)					
	350		450		550	
	Control	Experimental	Control	Experimental	Control	Experimental
Fish meal	510	510	660	660	810	810
Dextrin	272·5	272·5	160	160	70	70
Cod liver oil	46	46	34	34	10	10
Vitamin – mineral mix†	60	60	60	60	60	60
α-Cellulose	91·5	91·5	66	66	33	33
Binder‡	20	20	20	20	20	20
Gross energy (kJ/kg)‖	14600	14600	14900	14900	15100	15100
Proximate analysis (g/kg)§						
Protein	322	323	436	433	537	531
Fat	79	84	91	92	83	84
Fibre	120	115	80	83	50	50
Moisture	46	18	27	17	28	32
Ash	141	153	187	176	202	215
Carbohydrate (N-free extract)	338	325	206	216	128	120
Gross energy (kJ/kg)‖	13700	13700	14000	14150	14200	14000

* All diets contained 10 g chromic oxide/kg diet.
† Obtained from Edward Baker Ltd, Sudbury, Suffolk.
‡ Carboxy methyl cellulose.
‖ Calculated from (kJ/g): carbohydrate 15·75; protein 16·8; fat 37·8. (Cowey & Sargent, 1972).
§ According to the Association of Official Analytical Chemists (1975).

ingredients were blended in an Hobart mixer, and divided into two equal portions. To one portion (experimental diet) was added an ethanolic solution of ethylestrenol (17α-ethyl-19-norandrost-4-en-17β-ol; Maxibolin; Organon, USA) at a concentration of 3·5 mg/kg diet. An equivalent volume of ethanol, with no steroid, was added to the other portion (control diet). To 1 kg of both the control and experimental diets was added 10 g chromic oxide (Cr_2O_3). After extrusion from the mixer, the diets were air-dried, broken into pellets, and stored at −20° in sealed polyethylene bags.

On weekdays, trout were fed twice daily to satiation (10.00 and 16.00 hours), and at weekends, once daily (10.00 hours), with a record of food intake kept for each group. The control and experimental diets were given for 60 d, after which time, a commercial diet with no steroid, was given for a further 30 d. The composition of the diet given during this withdrawal period (Omega no. 6) was (g/kg): oil 60; protein 410; fibre 45; ash 105; moisture 90; carbohydrate (N-free extract) 290 (manufacturer's analysis).

Measurement of fish weight and length

At the start of the experiment, and thereafter every 15 d for 60 d, and after 90 d, trout were weighed and measured individually under light benzocaine (BDH) anaesthesia (1 ml saturated solution of benzocaine in 5 l water). Fish were weighed to the nearest 0·01 g, and total length measured to the nearest 1 mm. Food was witheld on the day of weighing and measuring.

Chemical analyses and tissue histology

Five fish were taken from each group at random on days 0, 30 and 60, and the liver, kidney, spleen, whole gut, brain and carcass of each fish weighed. Muscle tissue was stored frozen at −20° prior to analysis of moisture, protein and fat content (Association of Official Analytical Chemists, 1975). After 30 and 60 d, faeces, pooled from five fish, were taken for the determination of Cr_2O_3, N and fat content on three replicates of each pooled sample. Chromic oxide in food and faeces was determined by the method of Furakawa & Tsukahara (1966). Tissues were fixed for histological examination in Bouin's for 24 h, embedded in paraffin wax, and 5 μm sections stained in haematoxylin and eosin.

Analysis of results

The specific growth rate (SGR) was calculated according to Huisman (1976), and the condition factor, k (index of leanness or fatness), from the formula, $k = (W/L^3) \times 100$, where W is weight (g) and L is length (mm). FCE was taken as live-weight gain per unit weight of food consumed, and PER as live-weight gain per unit of protein consumed (Cowey & Sargent, 1972). The apparent digestibility of protein was determined according to Jobling (1981), and protein assimilation, according to Cowey & Sargent (1972). Tissue-somatic indices were taken as the ratio, wet tissue weight: fish weight, and expressed as a percentage. All results were analysed by the single-factor analysis of variance (ANOVA) according to Sokal & Rohlf (1969), using the 5% significance level.

RESULTS

Weight, length, SGR and k

After 60 d, the weight and length of the 430 and 530 g protein/kg controls were significantly greater than the 320 g protein/kg controls (Table 2) ($P < 0·01$ for weight and length). These changes were also reflected in higher SGR and k values (Table 3). Inclusion of steroid resulted in an increase in weight and length of the 320 and 430 g protein/kg experimental groups over the respective controls after 60 d ($P < 0·001$), and over the 60 d period, a higher SGR. During the steroid withdrawal period (60–90 d), a more marked effect was apparent in trout previously given the 320 g protein/kg experimental diet. The differences between

Table 2. *Effect of dietary protein level, ethylestrenol and steroid withdrawal, on body-weight (g) and length (mm) of rainbow trout (Salmo gairdneri)**

(Mean values with their standard errors for thirty-five fish/group at day 0, and twenty-five fish/group at days 60 and 90)

Protein level‡ (g/kg diet)	Period of experiment (d)												
	0				60†				90				
	Body-wt		Length		Body-wt		Length		Body-wt		Length		Total mortalities
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	
320 Control	28·29	0·65	134·0	0·9	75·71	1·92	179·4	1·4	106·65	3·61	198·1	1·9	1
320 Experimental	27·18	0·79	132·0	1·2	89·14	3·07	189·0	2·3	125·29	5·69	208·2	3·0	1
430 Control	26·64	0·71	131·0	1·0	86·33	2·86	184·0	2·0	111·27	4·58	201·4	2·6	0
430 Experimental	26·64	0·99	131·0	1·7	100·19	3·95	191·5	2·8	121·11	6·48	205·5	3·5	1
530 Control	28·62	0·67	134·0	1·2	86·30	2·40	185·3	2·0	111·27	3·77	201·2	2·1	0
530 Experimental	28·32	0·82	133·0	0·6	88·84	3·26	186·8	2·3	112·86	3·96	202·9	2·5	4

* Statistically significant differences (ANOVA). For weight at 60 d: 320 g protein/kg control *v.* experimental $P < 0·001$; 430 g protein/kg control *v.* experimental $P < 0·001$; 320 g protein/kg control *v.* 430 and 530 g protein/kg controls $P < 0·01$. For weight at 90 d: 320 g protein/kg control *v.* experimental $P < 0·05$. For length at 60 d: 320 g protein/kg control *v.* experimental $P < 0·001$; 430 g protein/kg control *v.* experimental $P < 0·01$; 320 g protein/kg control *v.* 530 g protein/kg control $P < 0·01$. For length at 90 d: 320 g protein/kg control *v.* experimental $P < 0·01$.

† Steroid withdrawn after 60 d and all trout given a steroid-free, commercial diet (Omega no. 6: 410 g protein/kg) for a further 30 d.

‡ Experimental diets contain ethylestrenol at 3·5 mg/kg diet. For details of diets, see Table 1.

Table 3. *Effect of dietary protein level, ethylestrenol and steroid withdrawal on the specific growth rate (for weight and length) and condition factor of rainbow trout* (Salmo gairdneri)*

(Mean values for thirty-five fish/group at day 0, and twenty-five fish/group at days 60 and 90)

Protein level† (g/kg diet)	Specific growth rate Period of experiment (d)						Condition factor Period of experiment (d)		
	0–60		60–90		0–90		0	60	90
	Body-wt	Length	Body-wt	Length	Body-wt	Length			
320 Control	1·64	0·49	1·14	0·33	1·47	0·43	1·17	1·31	1·37
320 Experimental	1·98	0·60	1·13	0·32	1·70	0·51	1·18	1·32	1·39
430 Control	1·96	0·57	0·85	0·30	1·59	0·48	1·18	1·39	1·36
430 Experimental	2·21	0·63	0·63	0·24	1·68	0·50	1·18	1·43	1·40
530 Control	1·84	0·54	0·85	0·27	1·51	0·45	1·18	1·36	1·37
530 Experimental	1·91	0·57	0·80	0·28	1·54	0·47	1·20	1·36	1·35

* Specific growth rate calculated from the mean initial and final weight and length of each group, for each period of the experiment, and is given as the percentage increase per unit time (d). Condition factor calculated from the mean initial and final weight and length of each group at day 0, and after 60 and 90 d.

† For details, see Tables 1 and 2.

Table 4. *Effect of dietary protein level and ethylestrenol on food consumption, food conversion efficiency and protein efficiency ratio of rainbow trout* (Salmo gairdneri)*

(Mean values/group, normalized to thirty fish/group)

	Protein level (g/kg diet)†					
	320		430		530	
	Control	Experimental	Control	Experimental	Control	Experimental
Total wt gain (g)	1595·70	2025·00	1922·10	2355·60	1886·10	2006·70
Wt gain over controls (g)	—	429·30	—	433·50	—	120·60
Total food consumption (g)	2515·56	2865·00	2446·55	2765·15	2405·00	2243·33
Total protein consumed (g)	804·98	916·80	1052·02	1189·01	1274·65	1188·96
Average daily ration (g)	41·93	47·75	40·78	46·09	40·08	37·39
Average daily intake (% body-wt)	1·85	1·79	1·57	1·53	1·55	1·40
Food conversion efficiency	0·63	0·70	0·78	0·85	0·78	0·89
Protein efficiency ratio	1·98	2·21	1·83	1·98	1·48	1·69

* All results refer to the 60 d period of steroid treatment.

† For details, see Tables 1 and 2.

the 320 g protein/kg control and experimental fish after 90 d were significant, both for weight ($P < 0·05$) and length ($P < 0·01$). The values for k indicated that ethylestrenol was more effective in increasing weight than length.

FCE and PER

Total and average per unit weight food consumption decrease in the controls with increasing dietary protein over 60 d (Table 4). Although total food consumption over 60 d was higher in the 320 and 430 g protein/kg experimental groups, and lower in the

Table 5. *Effect of dietary protein level and ethylestrenol on protein digestibility and assimilation, fat assimilation, and faecal nitrogen and fat content of rainbow trout* (Salmo gairdneri)

(Mean values of three replicates on a pooled sample from five fish/group at each time)

	Period of experiment (d)†	Protein level (g/kg)*					
		320		430		530	
		Control	Experimental	Control	Experimental	Control	Experimental
Apparent digestibility‡	30	0·72	0·83	0·79	0·84	0·75	0·87
	60	0·86	0·86	0·80	0·85	0·84	0·83
Facecal nitrogen (g/kg)	30	17·60	12·70	24·20	20·70	40·60	30·90
	60	12·00	12·90	25·10	21·20	29·60	27·00
Faecal fat (g/kg)	30	12·10	12·30	21·70	20·00	25·70	16·40
	60	8·20	11·00	7·20	28·10	6·60	16·60
Fat assimilation‖	30	0·85	0·85	0·76	0·78	0·69	0·80
	60	0·90	0·87	0·92	0·69	0·92	0·80
Protein assimilation§	30	0·65	0·75	0·64	0·70	0·52	0·64
	60	0·76	0·75	0·64	0·69	0·65	0·68

* For details, see Tables 1 and 2.
† Period of steroid treatment.
‡ Based on chromic oxide content in food and faeces.
‖ Based on food and faecal fat.
§ Based on food and faecal N.

Table 6. *Effect of dietary protein level and ethylestrenol on moisture, protein and fat content (g/kg) of muscle in rainbow trout* (Salmo gairdneri)*

(Mean values with their standard errors for five fish/group at each time)

Protein level‡ (g/kg diet)	Moisture				Protein				Fat			
	Period of experiment (d)†				Period of experiment (d)†				Period of experiment (d)†			
	30		60		30		60		30		60	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
320 Control	736·7	3·5	736·3	1·2	131·2	3·8	163·6	2·8	92·9	7·1	95·7	2·3
320 Experimental	743·8	2·7	725·2	3·5	147·6	2·9	175·5	1·2	93·2	7·1	91·2	0·8
430 Control	735·2	3·9	733·1	1·2	170·8	14·6	169·3	2·4	97·8	6·2	87·9	4·3
430 Experimental	734·2	4·9	729·0	3·1	164·7	6·3	171·3	3·3	94·2	7·3	96·9	3·9
530 Control	748·1	7·2	735·6	2·4	154·2	4·5	165·7	3·2	60·2	2·7	88·3	11·2
530 Experimental	722·8	3·3	727·4	3·1	203·3	14·7	174·0	1·4	66·8	8·5	93·6	8·7

* Statistically significant differences (ANOVA). 320 g protein/kg group for protein: 30 d control *v.* experimental $P < 0·01$; 30 d control *v.* 60 d control $P < 0·001$. 530 g protein/kg group for protein: 30 d control *v.* experimental $P < 0·02$. Between groups for protein: 320 g protein/kg control *v.* 430 g protein/kg control $P < 0·05$. Between groups for fat at 30 d: 320 and 430 g protein/kg controls and experimentals *v.* 530 g protein/kg controls and experimentals $P < 0·01$.
† Period of steroid treatment.
‡ For details, see Tables 1 and 2.

Table 7. *Effect of dietary protein level and ethylestrenol on tissue-somatic indices of rainbow trout* (Salmo gairdneri)*

(Mean values with their standard errors for five fish/group at each time)

Protein level‡ (g/kg diet)	Hepato-somatic index (HSI) Period of experiment (d)†				Reno-somatic index (RSI) Period of experiment (d)†				Viscero-somatic index (VSI) Period of experiment (d)†			
	30		60		30		60		30		60	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
320 Control	1·44	0·05	1·42	0·02	0·74	0·04	0·73	0·05	8·69	0·27	8·45	0·35
320 Experimental	1·12	0·06	1·12	0·07	0·93	0·05	0·94	0·08	7·64	0·24	5·74	0·30
430 Control	1·52	0·11	1·70	0·15	0·89	0·11	0·81	0·10	9·96	0·47	9·00	0·45
430 Experimental	1·16	0·07	1·31	0·06	0·98	0·04	1·07	0·05	7·52	0·28	5·88	0·42
530 Control	1·59	0·11	1·72	0·05	0·87	0·07	0·73	0·04	10·84	0·63	9·08	0·30
530 Experimental	1·38	0·14	1·33	0·02	1·01	0·14	1·11	0·03	7·46	0·62	5·68	0·36

* Statistically significant differences (ANOVA). HSI at 30 d: 320 g protein/kg control *v.* 430 and 530 g protein/kg controls $P < 0·05$; 430 g protein/kg control *v.* experimental $P < 0·001$. HSI at 60 d: 320 g protein/kg control *v.* 430 and 530 g protein/kg controls $P < 0·05$ and $P < 0·01$, respectively; 320 g protein/kg control *v.* experimental $P < 0·01$; 430 g protein/kg control *v.* experimental $P < 0·001$; 530 g protein/kg control *v.* experimental $P < 0·001$. RSI at 60 d: 320 g protein/kg control *v.* experimental $P < 0·05$; 430 g protein/kg control *v.* experimental $P < 0·01$; 530 g protein/kg control *v.* experimental $P < 0·001$. VSI at 30 d: 320 g protein/kg control *v.* 530 g protein/kg control $P < 0·01$; 430 and 530 g protein/kg controls *v.* experimentals $P < 0·001$. VSI at 60 d: 320, 430 and 530 g protein/kg experimentals *v.* respective controls $P < 0·001$.

† Period of steroid treatment.

‡ For details, see Tables 1 and 2.

530 g protein/kg group than the controls, average per unit weight intake was less in fish given steroid. In both control and experimental groups, PER decreased and FCE increased with increasing dietary protein content, but at each level, FCE and PER were higher in fish given steroid.

Apparent digestibility and assimilation

Calculated on the basis of Cr_2O_3 concentration in food and faeces, the apparent digestibility of dietary protein in the controls was variable, but was higher in fish given steroid after 30 d, and in the 430 g protein/kg experimental group, after 60 d (Table 5). In the controls, an increase in dietary protein resulted in a decrease in protein assimilation, and an increase in faecal N content, after 30 and 60 d. The effect of ethylestrenol was more evident after 30 d, when at each level of dietary protein, protein assimilation was higher, and faecal N content, lower than the controls.

Fat assimilation in control groups decreased with increasing dietary protein after 30 d, whilst faecal fat content increased. After 60 d, however, faecal fat of the controls was reduced, and fat assimilation increased. An increase in fat assimilation, and a reduced faecal fat content with increasing dietary protein, was also apparent in the controls after this time. In fish given steroid, faecal fat content was similar after 30 and 60 d, but higher than the respective controls after 60 d.

Muscle moisture, protein and fat

No significant changes in muscle moisture were seen in any of the groups over 60 d (Table 6). In the controls, muscle protein content was significantly higher in the 430 g protein/kg group than the 320 g protein/kg group ($P < 0·05$) after 30 d. However, the greatest increase

in muscle protein during the 30–60 d period occurred in the 320 g protein/kg control group ($P < 0.001$). There were no significant differences in muscle protein content of control fish after 60 d. In fish given steroid, a significant increase in muscle protein over respective controls occurred after 30 d in the 320 and 530 g protein/kg experimental groups ($P < 0.01$ and $P < 0.02$, respectively), but no differences were seen after 60 d. Muscle fat content in the 530 g protein/kg control and experimental groups was significantly lower after 30 d when compared with the other groups ($P < 0.001$), but no effects of ethylestrenol were apparent at any time.

Tissue-somatic indices

A significant increase in hepato-somatic index (HSI) occurred with increasing dietary protein in the controls after 30 and 60 d ($P < 0.05$), and at both time intervals, HSI in experimental fish was significantly lower ($P < 0.001$) (Table 7). Steroid treatment also resulted in a significant reduction in viscero-somatic index (VSI) after 30 d ($P < 0.01$) and 60 d ($P < 0.001$) in fish given steroid. The reno-somatic index (RSI) of control fish was similar after 30 and 60 d, but higher in the experimentals after 60 d, the greatest effect occurring in the 530 g protein/kg experimental group ($P < 0.001$). Histological examination of the kidneys revealed that the cells lining the tubules were consistently more columnar in fish given steroid. No significant changes in the relative weights of spleen, brain, gonads or carcass were found in any of the groups after 30 or 60 d (results not shown).

DISCUSSION

In agreement with previous studies in salmonids (Cowey & Sargent, 1979), the present results have shown that increasing the dietary protein level to between 400 and 500 g protein/kg, promotes a faster growth rate of trout (weight and length), and results in an increased FCE. The higher SGR and k of fish given ethylestrenol, is also in accord with earlier observations of ethylestrenol effects on trout growth (Simpson, 1976), and of anabolic-androgenic steroids in other teleosts (Donaldson *et al.* 1979; Matty & Lone, 1979; Lone & Matty, 1980*a*). The magnitude and duration of the steroid effects observed in the present study, appeared to be related to the dietary protein level. Growth rate was highest over 90 d in the group given a steroid-supplemented diet for 60 d, containing the lowest level of protein (320 g protein/kg experimentals).

The reduction in food intake with increasing dietary protein, observed in control and experimental groups, suggests that a high level of protein limits the ability of trout to increase their food intake to obtain more protein. These results, however, are insufficient to identify the possible factor(s), other than protein, which may have regulated this response. Nevertheless, Lee & Putnam (1973) concluded that energy content regulates food intake in trout, and found a positive correlation between the protein:energy value and percentage energy retention. An inverse relationship between food intake and dietary protein level is also evident from their results. Recent studies in rats have likewise shown food intake to be a function of dietary protein (Preston Mercer *et al.* 1981).

In trout given steroid, the higher total food intake observed is not consistent with the view that steroid treatment increases appetite in fish (Fagerlund *et al.* 1979; Yu *et al.* 1979). On an average per unit weight basis, food intake was lower in fish given steroid than the controls, indicating that a higher total food intake in this instance, was more a manifestation of increased growth. If the hypothesis of Lee & Putnam (1973) proves to be correct, then it must be concluded that ethylestrenol acts partly to enhance the efficiency of energy utilization. This response, taken with the increased FCE and PER of trout given steroid, has obvious commercial implications.

It has been suggested that a depression in the digestibility of low protein diets may be

due to the increased proportion of carbohydrate and fibre (Cowey, 1975; Cowey & Sargent, 1972, 1979). In the present study, despite variations in these constituents, no obvious pattern of effect on apparent digestibility was observed. However, with increasing dietary protein, protein assimilation decreased and faecal nitrogen content increased, suggesting a reduced deposition of protein when given at above optimal levels.

Evidence that ethylestrenol influences gastrointestinal function, is seen from the increased apparent digestibility and assimilation of protein over the controls, and the reduced faecal N content. These effects were more marked after 30 d, suggesting that the efficacy of steroid action diminished with time. Significant effects of androgenic steroids on gut histology and proteolytic activity, have been reported previously for trout (Yamazaki, 1976), and carp (Lone & Matty, 1981).

The changes in faecal fat content and fat assimilation are of particular interest in view of the constancy of this component in the diets. The control values revealed a relationship between faecal fat content and dietary protein level, and the opposing trends observed after 30 and 60 d, an apparent adaptatory response in relation to time. The high faecal fat content of the 530 g protein/kg controls after 30 d, taken with the significantly lower muscle fat levels at this time, suggest, that in the short term, when availability is above optimal levels, dietary protein is utilized for energy purposes in preference to fat. In trout given ethylestrenol, one possible consequence of the higher faecal fat content and lower fat assimilation after 60 d, would be a reduction in the potential energy contribution of fat, although no effects on muscle fat content were observed.

Muscle protein content was predictably higher in the 430 and 530 g protein/kg controls, and although increased after 30 d in the experimental groups, no effects were apparent after 60 d. Steroid-induced weight gains resulting from increased water retention, can be excluded as a possibility in this study, a finding in agreement with previous observations (Fagerlund *et al.* 1979; Fagerlund *et al.* 1980).

It has generally been accepted that diets high in carbohydrate result in increased relative liver weight (Phillips, 1969). In the present study, however, HSI was highest in trout given diets containing the lowest carbohydrate content (530 g protein/kg group). The same response was also noted by Lee & Putnam (1973) for trout given diets containing 80 g lipid/kg, but varying in carbohydrate and protein content. They further observed that with increasing dietary protein, the percentage liver lipid content decreased, and liver sugar increased, suggesting that in response to the higher levels of ingested protein, the rate of gluconeogenesis is enhanced. This adaptive capacity of trout is now well established (Cowey *et al.* 1977).

In agreement with studies in carp (Lone & Matty, 1980*a*, *b*), HSI of trout given ethylestrenol was significantly reduced. However, an increase or no change in HSI, in response to androgenic steroids, has also been found (Simpson, 1976; Matty & Cheema, 1978; Donaldson *et al.* 1979). The significant reduction in VSI of trout given steroid agrees with the observation of Simpson (1976), who suggested that the response may be the result of increased mobilization of perivisceral fat deposits. Lee & Putnam (1973) found relative gut weight to be more a function of dietary fat content in trout, and observed no differences in trout given varying levels of dietary protein. Similarly in the present study, no differences in relative gut weight were seen after 60 d, although a significant increase did occur with increasing dietary protein after 30 d. In mammals, androgenic steroids act not only on male accessory sex organs but also on many other tissues, one of the more responsive of which is the kidney (Kochakian, 1977). The significant increase in RSI, and the histological changes observed in response to ethylestrenol in the present study, and to other androgenic steroids in trout (Matty & Cheema, 1978), indicates a similar kidney sensitivity to steroid action in fish and mammals.

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