THE EFFECTS OF DIETARY SUPPLEMENTS
ON JUVENILE SALMONID GROWTH AND
PLASMA GROWTH HORMONE

by

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The effects of dietary supplements on juvenile salmonid growth and

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ABSTRACT

Growth hormone (GH) concentrations in the plasma of two species of juvenile salmonids were determined before and after four compounds were administered by either: a) dorsal aortic cannula (rainbow trout) or; b) incorporated with commercial fishfeed (coho salmon). The compounds used were: 1) branched chain amino acids (BCAA); 2) arginine aspartate (AA); 3) gamma-aminobutyric acid (GABA) and 4) clonidine. Coho salmon juveniles were examined for increases in overall growth (weight and length) and relationships between GH concentrations and size.

Plasma growth hormone concentrations in cannulated trout were increased after injection of either BCAA (212%) or GABA (862%) while AA or clonidine had no significant effect.

Coho yearlings fed any of the supplements for 56 days showed no significant increases in either weight or length, but plasma GH was increased by BCAA, GABA or clonidine. Yearlings given BCAA had significantly higher plasma GH levels after 42 days (264%). GABA treated yearlings showed elevated plasma GH after only 28 days (115%). The response to clonidine was more immediate with plasma GH increasing after 14 days (136%). There was a significant relationship between GH versus weight and length using the AA supplement, with the smaller fish appearing to have relatively greater concentrations of circulating plasma GH.

Coho salmon fry fed with any of the supplements for 70

days significantly increased in both weight and length. BCAA produced the largest increase in weight (52%) and length (13%), while AA produced the smallest increase in weight (31%) and length (8%).

Circulating plasma GH can evidently be elevated by direct injection of BCAA and GABA as well as after supplemented feedings with BCAA, GABA and clonidine. All four compounds increased weight and length of coho fry. One or more of these compounds might be used commercially as dietary growth promoters and should be investigated further.

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1.0 Introduction

Growth represents the net product of a series of physiological and behavioral processes which begin with food consumption and end in the deposition of tissue (Brett, 1979). Weatherley and Gill (1987) describe growth in the context used by fish biologists, as the increase in magnitude, measured or observed as increases in length, weight, or volume. Ricker (1979) considered the whole life cycle in dividing growth into stanzas or stages in development, such as maturation or changes in habitat or habits.

The growth of salmonids depends on a variety of exogenous and endogenous factors. These include the influences of genetics (McKay et al., 1986a,b), nutrition (Fagerlund et al., 1983; Higgs et al., 1985; Matty, 1986) and hormones (Higgs et al., 1975, 1976, 1977, 1982; Komourdjian et al., 1976; Clarke et al., 1977; Ludwig et al., 1977; Markert et al., 1977; Donaldson et al., 1979; Weatherley et al., 1980; Fagerlund et al., 1983; Matty, 1986; Ostrowski and Garling, 1986) as well as environmental parameters (Weatherley, 1976; Brett, 1979; Higgs et al., 1985; McKay and Gjerde, 1985; Siitonen, 1986).

In aquaculture, maximal growth at a minimal cost is desirable. Growth rates and feed utilization efficiencies become the most economically important aspects of raising salmonids (Markert et al., 1977; Matty, 1986). In recent

years, the possibility of using growth hormone (GH) to increase growth in cultured salmonids has received great interest (Higgs et al., 1975; 1976, 1977; Clarke et al., 1977; Markert et al., 1977; Donaldson et al., 1979) and its potential as a practical growth promoter has been investigated (Markert et al., 1977).

Vertebrate GH or somatotropin is a straight chain protein hormone of approximately 23,000 daltons molecular weight. Its production, storage and release occurs in specific cells called somatotrops located in the pars distalis region of the anterior pituitary gland (Martin, 1985). The name "growth hormone" was derived from its primary function in controlling somatic growth (Martin, 1985) and in mammals its effect is influenced and mediated by peptides called somatomedins (Scanes and Lauterio, 1984).

Growth hormone is thought to promote and control growth by increasing transport of free amino acids into cells and their subsequent incorporation into structural proteins, and causing an increased level of cytoplasmic mRNA (Gorbman et al., 1983). Other functions of GH, some of which may influence animal growth rates include: increasing appetite, perhaps by directly stimulating the hypothalamus; elevating metabolic rate; maintaining the immune system by playing a role in the primary antibody response; and interacting with other hormones to regulate carbohydrate, protein, lipid, nucleic acid, water, and electrolyte metabolism (Gorbman et al., 1983; Scanes and Lauterio, 1984; Martin, 1985; Hall et

al., 1986).

Some of these characteristics have made exogenous treatments with mammalian GH (ie. injections) beneficial in increasing the growth of meat-producing farm animals (Hart and Johnsson, 1986). Machlin (1972) used daily injections of porcine GH (pGH) to increase the daily weight gain in young pigs by up to 16% and improved their food conversion efficiency by 19%. Pigs also had significantly lower backfat thickness (20%), leading to the conclusion that GH plays an important role in fat metabolism. In sheep, the primary effect of ovine GH (oGH) injections was a decrease body fats (Hart and Johnsson, 1986). Johnsson et al. (1985) indicated that daily injections of bovine GH (bGH) promoted both weight gain (22%) and food conversion efficiency (12%) in sheep after 12 weeks. Chronic exogenous treatment of bGH to young dairy cattle increased the daily weight gain over 14 weeks by 13%, but food conversion efficiency was not examined (Sejrsen et al., 1983). Thus, in mammals exogenous GH treatment can be used to promote an increase in weight (meat) and increase the efficiency by which food is utilized.

Knowledge of the role of GH in salmonid growth and metabolism is limited, and much of the information available is from investigations with mammalian GH, primarily; bovine (bGH), ovine (oGH) and porcine (pGH) and the use of heterologous assays to measure serum levels.

Early studies with salmonids showed that exogenous

treatments with bGH increased growth and influenced tissue composition in both yearling (Higgs et al., 1975) and underyearling (Higgs et al., 1976) coho salmon (Oncorhynchus kisutch). In yearling coho, fish either injected intraperitonealy (i.p.) or pellet implanted with 10 or 100 ug/g body weight of bGH grew more than controls. Those fish treated with the bGH weighed 40 to 66% more 8 weeks after treatment, than controls. Administration of bGH also appeared to have a protein anabolic or anticatabolic action by increasing the total amount of body protein by 35 to 59% in treated fish. At the same time bGH lowered the muscle lipid and lipid to dry weight ratio indicating a possible role in lipid metabolism.

Underyearling coho salmon, treated with 10 or 30 ug/g body weight every week also grew significantly faster compared to controls. Gains in weight and length in most groups occurred after only 14 days of treatment and at the end of the 84 day experiment the size of treated fish exceeded controls by 220 to 369%. Minimum dosage needed to maximize the growth potential of these underyearling coho was only 10 ug/g body weight/week. In addition, as in coho yearlings treated with bGH, the percentage of muscle protein increased while muscle lipid and lipid to dry body weight also decreased. Donaldson et al. (1979) noted that although both yearling and underyearling coho grew faster, the underyearlings (Higgs et al., 1976) which would have the greatest growth potential due to their size and/or age

(Ricker, 1979) grew faster following bGH treatment than yearlings (Higgs et al., 1975).

Markert et al. (1977) injected (i.p.) coho yearlings with bGH and demonstrated that they not only grew faster, but also converted food more efficiently. In this study all fish were injected with 10 ug/g body weight weekly and the growth recorded over a 56 day period. The treated fish demonstrated better food and protein conversion, in addition to increased growth rate and lower condition factors, inducing greater growth in length than weight. They concluded that although treatment was successful, it was too expensive to be practical.

In the Atlantic salmon, Salmo salar, Komourdjian et al. (1976) injected (i.p.) parr with pGH (1.0 ug/g body weight) in saline. They were injected on alternate days over a 28 day period and the growth measured 4 times during the experiment. The results were similar to those found using coho salmon (Higgs et al., 1975, 1976), in that length significantly increased while condition factor decreased. In addition to the increase in growth, survival in seawater was significantly higher indicating a possible role of GH in seawater adaptation.

In addition to the studies on growth using mammalian GH, Clarke et al. (1977) showed that Tilapia (Tilapia mossambicus) GH promoted growth in juvenile sockeye salmon (O. nerka) and Higgs et al. (1978) showed that salmon pituitary extracts promoted growth in juvenile coho salmon.

Clarke et al. (1977) used newly purified Tilapia GH injected into juvenile sockeye salmon to increase both weight and length. Higgs et al.(1978) demonstrated the presence of a growth promoting factor in chinook salmon (O. tshawytscha) pituitary extract. The extracts were intramuscularly injected (i.m.) in yearling coho salmon once a week over a 70 day period and significantly increased growth rates.

Early investigations into the metabolic responses in salmonids to GH revealed that GH played an important role in fuel mobilization (ie. lipids) (McKeown and van Overbeeke, 1972; McKeown et al., 1975, 1976; Clarke, 1976; Sweeting et al., 1985; Sheridan, 1986). McKeown and van Overbeeke (1972) showed GH was possibly associated with mobilization of metabolic fuels in migrating sockeye salmon (O. nerka). Growth hormone concentrations decreased in the pituitary, but were unchanged in the plasma during migration. These results led to the hypothesis that circulating GH may play a role in the metabolic demands during spawning migration in Pacific salmon. To relate changes in fuel stores to GH concentrations, McKeown et al. (1975) injected oGH into exercised kokanee salmon (O. nerka) juveniles. The excercise regime significantly increased circulating GH over the swimming period. Injections of oGH decreased free fatty acids (FFA) in the muscle while increasing glycogen in the liver and a hyperglycemic state similar to that found in mammals. Further support to the metabolic importance of GH was provided by McKeown et al. (1976), Clarke (1976),

Sweeting et al. (1985) and Sheridan (1986). McKeown et al. (1976) demonstrated that the elevation of circulating GH by injection resulted in an increase in plasma FFA, and that during this period of elevated GH, plasma glucose was also elevated. In support of the hyperglycemic effect found by McKeown et al. (1976), Sweeting et al. (1985) elicited a similar response in coho salmon presmolts. Injections of oGH increased plasma glucose by approximately 200% and decreased in effectiveness with the onset of smolting. This may suggest the early mobilization of fuel reserves as a preparatory role to smolting. Clarke (1976) demonstrated a reduction in the total amount of body lipids in juvenile sockeye salmon following an injection of mammalian GH, indicating possible lipolytic actions of GH. Growth hormone implants have also been shown to stimulate lipid mobilization in coho salmon parr, while having no effect on smolts (Sheridan, 1986). Thus, Sheridan (1986) suggests that the effects of GH treatments vary with tissue type and developmental stage, further complicating the understanding of the role GH plays in metabolism. Therefore, although most of these early studies used mammalian GH and heterologous assays, the findings support a role for GH in the mobilization and manipulation of fuel deposits in the salmonid.

Recently, salmonid GH (sGH) has been isolated and purified from the genus, Oncorhynchus (Wagner and McKeown, 1983; Wagner et al., 1985). With the purification of sGH

further research developed and validated a homologous radioimmunoassay (RIA) for sGH (Bolton et al., 1986; Wagner and McKeown, 1986), aiding the investigation of the complex role GH plays in the metabolic processes and subsequent growth and adaptability of the salmonid (Sweeting et al., 1985; Bolton et al., 1987; Richman and Zaugg, 1987; Sweeting and McKeown, 1987; Barrett and McKeown 1988a,b,c).

In vertebrates, the release of GH is regulated by three hypothalamic peptide hormones, namely; somatostatin (SRIF), growth hormone releasing factor (GRF) and thyrotropin releasing hormone (TRH) (Fig. 1) (Scanes and Lauterio, 1984). Somatostatin, which is an inhibitory hormone, prevents the release of GH into the systemic circulatory system. Thus, by elevating SRIF, a decrease or lack of GH response is seen (Scanes and Lauterio, 1984; Sweeting and McKeown, 1986). Growth hormone releasing factor and TRH on the other hand , both increase the secretion of GH from the somatotrops (Scanes and Lauterio, 1984; Martin, 1985).

Primary control of GH secretion in the vertebrate pituitary appears to be controlled by the two major hypothalamic factors, GRF and SRIF (Murakami et al., 1987). These peptides may in turn be regulated by other hypothalamic monoamines and central transmitters (Arimura and Cullen, 1985; Hall et al., 1986).

In the rat the mediobasal and anterior hypothalamic regions that participate in the regulation of GH (Willoughby et al., 1986). The medial basal hypothalamus (MBH) and in

Figure 1. A schematic outlining the proposed control of growth hormone release and inhibition from the mammalian pituitary. NE/E=norepinephrine/epinephrine, 5HT=serotonin, T4=L-thyroxine, T3=L-triiodo-thyronine.

? Nutrients (−)
? Somatomedins (−)
Stress (−)

Hypothalamus

NE/E (+)
5HT (−)

SRIF (−) GRF (+) TRH (+)

Pituitary Gland

E/NE (+) ?
T4/T3 (−)

GH

Liver

Somatomedins

Lipid metabolism
Other endocrine functions ?

GROWTH
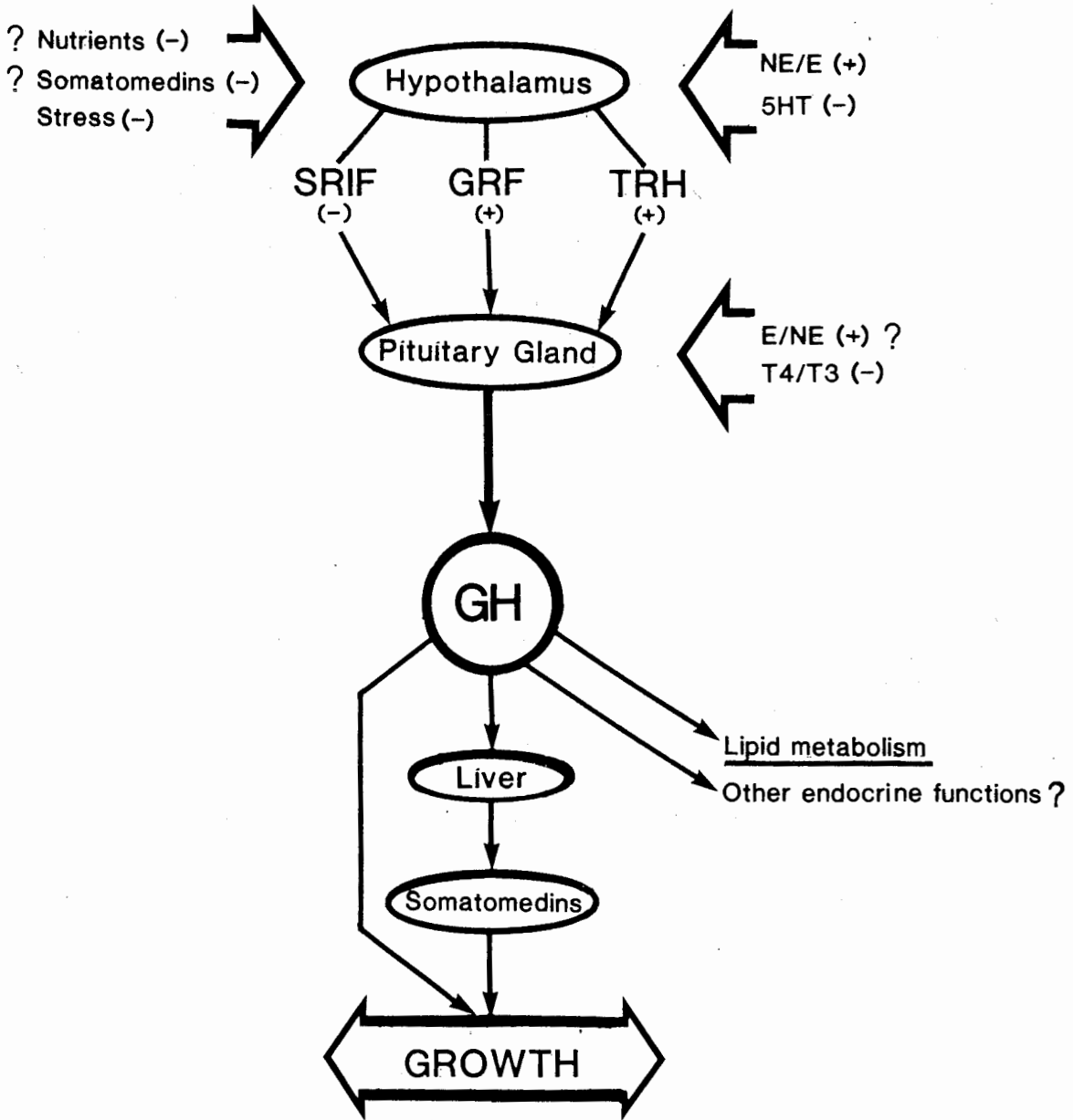
Adapted from: Scanes and Lauterio (1984)

particular the arcuate nucleus and ventromedial nucleus appear to be areas of primary importance in the stimulatory control of the central nervous system (CNS) on GH release (Muller, 1987). Additional hypothalamic regions such as the preoptic anterior hypothalamic area (PO/AHA) also appear to be important in stimulating GH secretion (Muller, 1987). The regions of the hypothalamus involved in GH regulation in salmonids are thought to be similar to those in the rat (Luo and McKeown, unpubl. observ.).

Factors other than the hypothalamic hormones also affect the release of GH in vertebrates and include such external stresses as restraint, temperature fluctuations, anaesthetics and food deprivation (Hall et al., 1986). In addition, such compounds as steroid hormones, some amino acids, glucose, fatty acids and inorganic ions (Hall et al., 1986) have also been shown to affect GH release in vertebrates (Hall et al., 1986).

The purpose of this study was to investigate whether some of the above compounds would influence plasma GH and growth in salmonids. Compounds selected for this study were: 1) the branched chain amino acids, valine, leucine and isoleucine; 2) arginine aspartate, the arginine salt of aspartic acid; 3) gamma-aminobutyric acid, a neurotransmitter and; 4) clonidine, a synthetic alpha-2-adrenergic agonist.

The branched chain amino acids (BCAA) are unique among the essential amino acids in that they are degraded to a large extent by extrahepatic tissues (Walton and Cowey,

1982; May et al.,1987). Branched chain amino acid levels
have also been shown to remain chronically elevated in
plasma during catabolic conditions such as starvation and in
subjects fed on diets high in protein (Harper, 1968; Kaushik
and Luquet, 1979; Stewart et al., 1984). Evidence by Adibi
(1980) and Fernstrom (1983) suggests that elevated levels of
plasma BCAA has an effect on both metabolic and neural
pathways by competing with precursor amino acids such as
tyrosine and tryptophan for entry to the brain. Stewart et
al. (1984) found that infusing the BCAA's into the blood of
the baboon elevated GH concentration, thus an influence on
GH release via an unknown mechanism.

Arginine aspartate is an arginine salt of aspartic
acid. Arginine is one of the ten essential amino acids
required by salmonids (Rumsey and Ketola 1975; Ketola, 1982;
Walton and Cowey, 1982) and causes a variety of effects on
the endocrine system in mammals (Davis, 1972; Franchimont et
al., 1984), while little is known of the effects of aspartic
acid (Franchimont et al., 1984). In rats, Franchimont et al.
(1984) compared the complex of arginine aspartate to each of
the single amino acids tested separately and found it
resulted in the greatest increase in circulating GH levels.

Gamma-aminobutyric acid (GABA) is a neurotransmitter
that appears to influence GH secretion in mammals (Hall et
al., 1986). It is produced by the decarboxylation of the
amino acid glutamic acid and is believed to be accumulated
in both the neurons and glia (Iversen et al., 1973; Acs et

al., 1984). Results from studies with the rat have shown that GABA has a direct stimulatory effect on the pituitary and GH secretion, particularly during early life stages, and possibly operates directly on the hypothalamus releasing GRF (Acs et al., 1984).

Clonidine, an adrenergic agonist, is used in man as an anti-hypertensive drug and is a powerful stimulator of GH release in both man and rat (Lal et al., 1975; Gil-Ad et al., 1979; Slover et al., 1984; Hunt et al., 1986; Soderpalm et al., 1987). Eden et al. (1981) and Eriksson et al. (1982) both indicate that clonidine operates on the adrenoreceptors in the hypothalamus exerting a stimulatory effect on GRF release and subsequent GH increase.

These four compounds were selected for trial on the basis of two criteria, namely; 1) the cost, as expensive compounds would not be practical in large scale fish production; 2) the ease and safety of handling if and when the compounds were to be used in a production setting.

This study was composed of four individual experiments, one involving the direct injection each test compound into the circulatory and three involving the feeding of the compounds. The hypotheses to be tested were:

1) That the injection of BCAA, AA, GABA or clonidine directly into the circulatory system of rainbow trout would significantly increase plasma GH;

2) That plasma GH and growth (weight and length) would increase from long term feeding with BCAA, AA, GABA or

clonidine in yearling coho salmon;

3) That growth (weight and length) would increase from long term feeding with BCAA, AA, GABA or clonidine in coho salmon fry;

4) That the age or size of the salmonid would affect the response obtained from supplemental feeding with BCAA, AA, GABA or clonidine.

Generally, it was postulated that by stimulating the endogenous release of GH in juvenile salmonids, an increase in tissue growth and animal size may be possible. If such a compound did increase GH and subsequent growth, it might provide a simple diet based growth promoter for aquaculture.

2.0 MATERIALS AND METHODS

2.1 Experimental Animals

Rainbow trout, Salmo gairdneri (mean weight = 429 g) used in cannulation studies were obtained from the West Creek Trout Farm (Aldergrove, B.C.). All trout were transported to the Simon Fraser University (S.F.U.) aquarium facility and held indoors in a 2400 L fibreglass tank, supplied with aerated, dechlorinated freshwater and subjected to a simulated natural photoperiod (May-July, 1987). Fish were fed ad libitum every 48 hours with Ewos brand fishfood (Ewos Ltd., Surrey, B.C.) until 48 hours before the experiment.

Coho salmon, Oncorhynchus kisutch yearlings (age 1+) and fry (age 0+) were obtained at a mean weight of 19.93 g and 0.40 g respectively, from the Capilano River Salmon Hatchery (North Vancouver, B.C.). All salmon were transferred to the S.F.U. aquarium facility where they were acclimated in 120 L fibreglass tanks. Tanks were supplied with aerated, dechlorinated freshwater and the fish were placed on a simulated natural photoperiod (February-September, 1987). Coho yearlings were fed with Oregon Moist Pellets (O.M.P.) (Moore Clarke, LaConner, Wash.) at S.F.U. for one week then given Ewos brand fishfeed. Coho fry were given Ewos brand fishfeed immediately upon transfer to the S.F.U. aquarium facility. All coho were fed three to four times a day to satiation and at the end of the day the

excess feed and fecal material was removed to ensure minimal degradation of water quality.

2.2 Test Compounds

Branched chain amino acids (BCAA) (leucine, isoleucine and valine), arginine aspartate (AA), gamma-aminobutyric acid (GABA) and clonidine (Sigma Chemical Co.) were suspended in fish physiological saline (Wolf, 1963), and injected through a cannula into the dorsal aorta of rainbow trout. Control animals were injected with saline.

The same compounds were given to coho salmon with their feed. The compounds were mixed with 10 mls of Canola oil (Canbra Foods, Lethbridge, Alta.). The oil mixture was then added to fishfeed coating the pellets. Control diets had Canola oil added to the feed at the same volume (10 mls). Enough mixture was prepared for two weeks and stored in a refrigerator at 4 C.

To assess the adhesion of Canola oil and compound to the fishfeed, water samples were collected from a tank containing standing water with a sample of BCAA treated feed added. These samples were collected when the feed was first added, and at 5, 15 and 60 minutes later. Water samples were measured for suspended or dissolved BCAA using the HPLC techniques described in section 2.42.

2.3 Experiment 1: Trout Cannulations

Cannulae were placed into the dorsal aorta at the first gill arch using a modification of the technique described by

Soivio et al. (1975). Cannulae were constructed from 18 cm of intramedic polyethylene tubing (PE 50, Clay Adams). They were inserted surgically and sutured to the roof of the mouth while the fish were anaeasthetized in a tank containing bicarbonate buffered (pH 7.0) tricaine methane sulfonate (MS-222, 170 mg/L) (Syndel, Vancouver, B.C.). After cannulation, the fish were transferred to individual blackened plexiglass experimental chambers (10 L) where they were isolated from each other and allowed to recover from the cannulation procedure.

Compounds were suspended in fish physiological saline and then 500 ul were injected into each of 3 to 6 fish. Dosages selected were: BCAA; valine 12.0 mg/kg body weight, leucine 7.0 mg/kg body weight, isoleucine 5.0 mg/kg body weight; AA 250 mg/kg body weight; GABA 0.50 mg/kg body weight; and clonidine 0.25 mg/kg body weight. Control fish (3 to 6) were injected with 500 ul of saline.

Blood samples were collected from the cannulae before injections then 30, 90, and 210 minutes after. Each blood sample was 300 ul in volume and collected in 1 ml disposable syringes rinsed with heparin (150 IU/L) to prevent clotting. After collecting the blood sample, the cannula was flushed with 100-200 ul of saline. Samples were then centrifuged for 3 minutes at 1500g using a Model CL, International Clinical Centrifuge. Plasma was removed and placed in clean 1.5 ml microcentrifuge tubes, frozen on dry ice and stored at -20 C.

Growth hormone levels in the plasma from cannulated rainbow trout were measured using a double antibody homologous salmon radioimmunoassay (RIA). The protocol was that of Wagner and McKeown (1986) using purified chum salmon (O. keta) growth hormone as the standard and coho salmon growth hormone as the labelled antigen. Plasma samples were run on the RIA in duplicate 50 ul volumes and the GH concentration recorded in ng/ml.

2.4 Experiment 2: Treated Coho Yearlings

2.41 Growth and Growth Hormone

Coho yearlings were separated with a dipnet into 6 non replicated groups and assigned a tank using a completely randomized design. Group A, fed with BCAA (valine, leucine, isoleucine) (N=120); Group B, the control for Group A (N=118); Group C, fed with AA (N=50); Group D, fed with GABA (N=50); Group E, with fed clonidine (N=50); Group F, the control for Groups C, D and E (N=50). The concentration of test compounds fed with the food in Groups A,C, and D was selected at 3.8 mg/g total fish weight/two weeks. Group E was fed with the test compound at a rate of 0.08 mg/g total fish weight/two weeks. Feeding rates were based on the manufacturers recommendations and ranged from 1.5% to 5.0% of the total fish weight every day depending on the temperature of the rearing water. The mean starting weights and lengths (mean $\pm$ SEM) of the fish assigned to experimental groups were: Group A, 20.56 g $\pm$ 1.47 g and

125.7 mm ± 2.9 mm; Group B, 19.27 g ± 1.52 g and 123.9 mm ± 3.1 mm; Group C, 18.21 g ± 1.25 g and 125.8 mm ± 2.8 mm; Group D, 21.67 g ± 1.0 g and 131.9 mm ± 2.0 mm; Group E, 22.64 g ± 1.89 g and 135.8 mm ± 3.5 mm and Group F, 20.32 g ± 1.12 g and 130.4 mm ± 2.5 mm.

All groups were reared in 120 L fibreglass tanks supplied with flow through, aerated, dechlorinated freshwater at 6.0 l/min. The mean water temperature during the study with Groups A and B, was 5.0 C and ranged from 3.5 to 7.0 C. The mean water temperature during the study with Groups C, D, E and F was 14.7 C and ranged from 13.0 to 16.0 C.

Sampling was conducted every two weeks at which time a random sample of 8-10 individuals was removed from each group. Individuals were anaesthetized with buffered MS-222 (170 mg/L), blotted dry and weighed to the nearest 0.1 g on a Sartorius Model 1407 electronic balance. Nose to fork lengths were then recorded to the nearest 1.0 mm. The total duration of the experiment was 56 days.

Blood samples were collected by severing the caudal peduncle and applying a heparinized hematocrit tube to the caudal vessel. Samples were collected at the same time during the day to minimize variation between samples due to circadian rhythms (Bates et al., 1989). Blood samples were centrifuged and the plasma stored as previously described for the cannulated rainbow trout. Samples of 20-50 ul of plasma were run in duplicate on the RIA . Hematocrits (Hbt)

were determined for Groups A, B, C, D and E (N=5) on day 56.

2.42 Amino Acid Analysis

Amino acid analysis of Ewos brand fishfood, plasma samples from coho salmon fed with BCAA and water samples collected from a BCAA treated static water tank was conducted using reverse-phase high performance liquid chromatography (HPLC). A sample of 5 mg of fish food was digested by first adding 1 ml 6N HCl. The sample was then vacuum degassed and heated at 110 C for 24 hours. The slurry was filtered through a Millex-GS 0.22 um filter unit. Pooled plasma samples (week 8, BCAA) of 100 ul were deproteinated using 20% trichloroacetic acid (TCA). Water samples (20 ul) were filtered through a Millex-GS 0.22 um filter unit to remove particulate matter.

Supernatants from all three preparations were collected and 20 ul samples derivatized using the phenylisothiocyanate (PITC) (Pierce Chemical Co.) procedure described by Heinrikson and Meredith (1984). Samples were first dried under nitrogen gas and then resuspended in 100 ul of coupling buffer (10 mls acetonitrile: 5 mls pyridine: 2 mls triethylamine: 3 mls water). The sample was again dried and resuspended in coupling buffer. To the sample/buffer mixture the PITC derivatization agent (5 ul) was added and allowed to react for 5 minutes. Samples were again dried and resuspended in 250 ul of a water: acetonitrile buffer (7:2), a 10 ul sample of the final solution was then run on the

HPLC.

Amino acid analysis was conducted with a Waters HPLC system employing a C18 NOVA pak column. Total time required for each sample was 48 minutes with the solvent and solvent gradient outlined by Scholze (1985). Amino acids were measured in picomoles focusing on valine, leucine, isoleucine, arginine and aspartate.

2.5 Experiment 3: Treated Coho Fry

Coho fry were separated using a dipnet into 6 replicated groups and assigned to a tank using a completely randomized design: Group 1, fed with BCAA (valine, leucine, isoleucine) (N=300); Group 2, fed with AA (N=300); Group 3, fed with GABA (N=300); Group 4, fed with clonidine (N=300); Group 5, was fed Canola oil and was the control for Groups 1,2,3 and 4 (N=300); Group 6, control for Group 5 (N=300). The concentration of the test compounds was the same as that for the coho yearlings and the feeding rates were based on manufacturers recommendations and ranged from 5.0% to 6.0% of the total fish weight every day. The mean pooled weight and length at the start of this study was 0.44 g $\pm$ 0.03 g and 36.5 mm $\pm$ 0.6 mm (mean $\pm$ SEM).

Individual groups were placed into rectangular, flowthrough, 26 L raceways. Each raceway supplied with dechlorinated freshwater from a constant header tank at 0.4 l/min. and aerated. The mean water temperature during the study was 11 C and ranged from 9.5 to 11.5 C.

Sampling was conducted every two weeks by removing a random sample of 10 individuals from each group. Individuals were anaesthetized with buffered MS-222 (170 mg/L), measured as previously described for the coho yearlings.

2.6 Experiment 4: BCAA Concentrations

Coho fry were separated using a dipnet into 6 replicated groups and assigned a tank using a completely randomized design. Three groups were fed with BCAA in the ratio of 1:1:1 (val:leu:ile) and three groups at 2:2:1 (val:leu:ile). Concentrations of BCAA varied within the two ratios such that: Group I (N=100) was fed with 1.3 mg/g body weight at 1:1:1; Group II (N=100) was fed with 3.8 mg/g body weight at 1:1:1; Group III (N=100) was fed with 11.4 mg/g body weight at 1:1:1; Group IV (N=100) was fed with 1.3 mg/g body weight at 2:2:1; Group V (N=100) was fed with 3.8 mg/g body weight at 2:2:1 and Group VI was fed with 11.4 mg/g body weight at 2:2:1. These levels of BCAA were given with the feed as previously described. Feeding rates were based on manufacturers recommendations and varied from 3.0% to 4.2% of the total fish weight every day. The mean pooled size (mean $\pm$ SEM) at the start of the experiment was 1.51 g $\pm$ 0.07 g and 51.0 mm $\pm$ 1.0 mm.

Individual groups were placed into rectangular raceways as previously described with a water flow of 0.6 L/min. The mean water temperature during the study was 14.5 C and ranged from 13.0 to 16.0 C.

Individuals were sampled every two weeks using the same procedure previously described.

2.7 Statistical Analyses

All statistical analysis was preformed using the SPSSX data analysis system (SPSSX, 1986). Cannulation and growth data were analysed by two-way ANOVA in order to assess interactions between time and treatment. Homogeneity of the group variances were first tested using Bartlett's test and where applicable heterogeneity of the groups was reduced by logarithmic transformation (log10). Student's t-test was used to compare the mean concentrations found in the growth hormone assay and plasma BCAA results from yearling coho salmon. Significance in all tests was accepted at the 95% confidence level ($p \leq 0.05$).

Pearson correlations were performed on weight and length versus GH data for coho yearlings in experiment 2. Where a significant relationship existed a model II linear regression was used to describe the interaction. The level of significance was set at 95% ($p \leq 0.05$).

3.0 RESULTS

3.1 Experiment 1: Trout Cannulations

Results of the cannulae injections with BCAA showed plasma GH increasing significantly by 90 minutes and remaining elevated at 210 minutes after injection (Table I). No significant changes were found when the interactive effects of treatment and time were analysed at either 30 ($F=0.015$, $p=0.684$), 90 ($F=0.102$, $p=0.388$) or 210 minutes ($F=0.148$, $p=0.230$). The concentration of plasma GH in treated fish at 90 and 210 minutes exceeded the levels found in the controls by 212% and 194% respectively.

Cannulae injections with AA did not result in any significant changes in plasma GH during the 210 minutes after injection (Table II). There were no significant effects from the interaction of treatment and time at either 30 ($F=2.265$, $p=0.158$), 90 ($F=2.344$, $p=0.123$) or 210 minutes ($F=1.869$, $p=0.162$).

Results from the cannulae injections with GABA showed significant increases in plasma GH at 90 and 210 minutes after injections (Table III). No significant interactive effects between treatment and time were found at either 30 ($F=0.983$, $p=0.347$), 90 ($F=0.617$, $p=0.554$) or 210 minutes ($F=0.510$, $p=0.680$). The concentration of plasma GH in treated fish at 90 and 210 minutes exceeded the levels found in the controls by, 862% and 139% respectively.

Cannulae injections with clonidine did not result in

Table I. The concentration of plasma growth hormone in cannulated rainbow trout injected with BCAA or saline (control) over 210 minutes. Numbers are the mean of n fish $\pm$ standard error.

Time (minutes)	Growth Hormone (ng/ml)	
	Saline	BCAA
0	4.6 $\pm$ 1.3 n=5	4.4 $\pm$ 1.1 n=6
30	3.2 $\pm$ 0.7 n=4	4.6 $\pm$ 2.2 n=4 F=0.003 p=0.845
90	2.5 $\pm$ 0.6 n=5	7.8 $\pm$ 2.8 * n=5 F=4.367 p=0.042
210	3.6 $\pm$ 1.0 n=5	10.6 $\pm$ 2.6 * n=4 F=5.037 p=0.032

* significantly different from control

Table II. The concentration of plasma growth hormone in cannulated rainbow trout injected with arginine aspartate (AA) or saline (control) over 210 minutes. Numbers are the mean of n fish ± standard error.

Time (minutes)	Growth Hormone (ng/ml)	
	Saline	AA
0	5.1 ± 1.3 n=4	4.6 ± 0.9 n=4
30	5.8 ± 1.5 n=4	16.5 ± 7.2 n=4 F=1.609 p=0.229
90	12.2 ± 4.6 n=4	6.9 ± 2.4 n=5 F=0.038 p=0.848
210	43.2 ± 25.0 n=3	5.9 ± 1.2 n=4 F=0.508 p=0.483

* significantly different from control

Table III. The concentration of plasma growth hormone in cannulated rainbow trout injected with GABA or saline (control) over 210 minutes. Numbers are the mean of n fish $\pm$ standard error.

Time (minutes)	Growth Hormone (ng/ml)	
	Saline	GABA
0	6.3 $\pm$ 2.0 n=3	8.3 $\pm$ 0.9 n=3
30	6.8 $\pm$ 2.6 n=4	20.9 $\pm$ 10.1 n=3 F=4.710 p=0.058
90	3.4 $\pm$ 0.7 n=4	32.7 $\pm$ 26.7 * n=3 F=6.945 p=0.020
210	3.6 $\pm$ 1.0 n=3	8.6 $\pm$ 1.4 * n=3 F=10.551 p=0.004

* significantly different from control

any significant changes in plasma GH between the treated fish and controls fish (Table IV). The interactive effects of treatment and time were also found to be non significant at 30 (F=0.279, p=0.612), 90 (F=0.537, p=0.597) and 210 minutes (F=0.420, p=0.741).

3.2 Experiment 2: Treated Coho Yearlings

BCAA (Group A), AA (Group C), GABA (Group D) and clonidine (Group E) did not increase the growth of treated fish significantly over the controls (Groups B and F) during the 56 day period.

Figure 2 illustrates the average weights (F=0.652, p=0.422) and lengths (F=0.419, p=0.519) of fish fed with BCAA versus controls. Figure 3 illustrates the average weights (F=0.085, p=0.772) and lengths (F=0.205, p=0.652) of fish fed with AA versus controls. Figure 4 illustrates the average weights (F=1.429, p=0.236) and lengths (F=0.105, p=0.747) of fish fed with GABA versus controls. Figure 5 illustrates the average weights (F=0.204, p=0.649) and lengths (F=3.87, p=0.053) of fish fed with clonidine versus controls. There were no significant interactive effects of treatment and time on weight in either the BCAA (F=0.251, p=0.908), AA (F=0.936, p=0.448), GABA (F=0.635, p=0.639) or clonidine (F=0.751, p=0.561) groups. Similarly, there were no significant interactive effects of treatment and time on length in either the BCAA (F=0.305, p=0.874), AA (F=1.170, 0.332), GABA (F=0.146, p=0.964) or clonidine (F=0.870,

Table IV. The concentration of plasma growth hormone in cannulated rainbow trout injected with clonidine or saline (control) over 210 minutes. Numbers are the mean of n fish $\pm$ standard error.

Time (minutes)	Growth Hormone (ng/ml)	
	Saline	Clonidine
0	6.3 $\pm$ 2.0 n=3	6.4 $\pm$ 0.1 n=2
30	6.8 $\pm$ 2.6 n=4	13.9 $\pm$ 8.0 n=3 F=0.968 p=0.354
90	3.4 $\pm$ 0.7 n=4	14.9 $\pm$ 10.0 n=3 F=3.531 p=0.083
210	3.6 $\pm$ 1.0 n=3	6.6 $\pm$ 3.3 n=3 F=4.078 p=0.059

* significantly different from control

Figure 2. The average growth of coho salmon yearlings (age 1+) (n=10) fed BCAA (□) versus controls (○) reported as (A) mean weights (gm) and (B) mean lengths (mm) over 56 days. The vertical lines represent the SEM and were omitted if they overlapped.
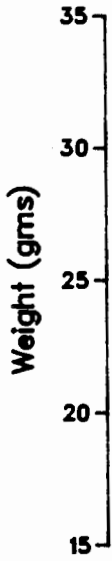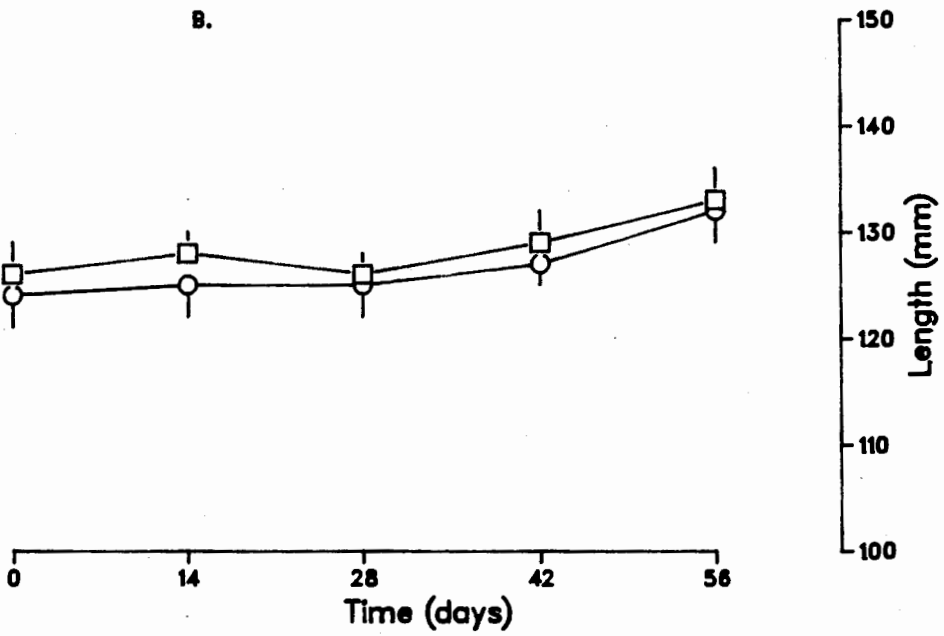
Figure 3. The average growth of coho salmon yearlings (age 1+; n=8) fed AA (□) versus control (○) reported as (A) mean weights (gm) and (B) mean lengths (mm) over 56 days. The vertical lines represent the SEM were omitted if they overlapped.
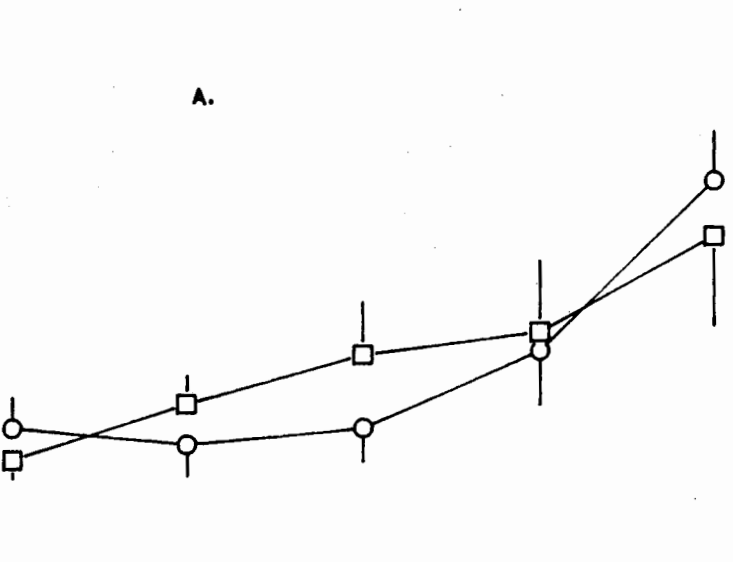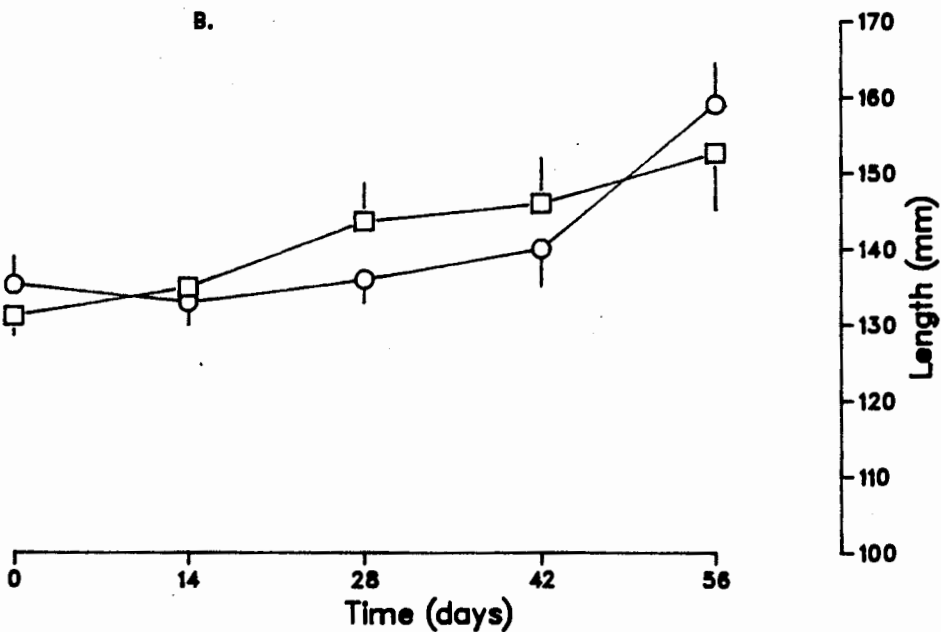
Figure 4. The average growth of coho salmon yearlings (age 1+) (n=8) fed GABA (□) versus controls (O) reported as (A) mean weights (gm) and (B) mean lengths (mm) over 56 days. The vertical lines represent the SEM and were omitted if they overlapped.
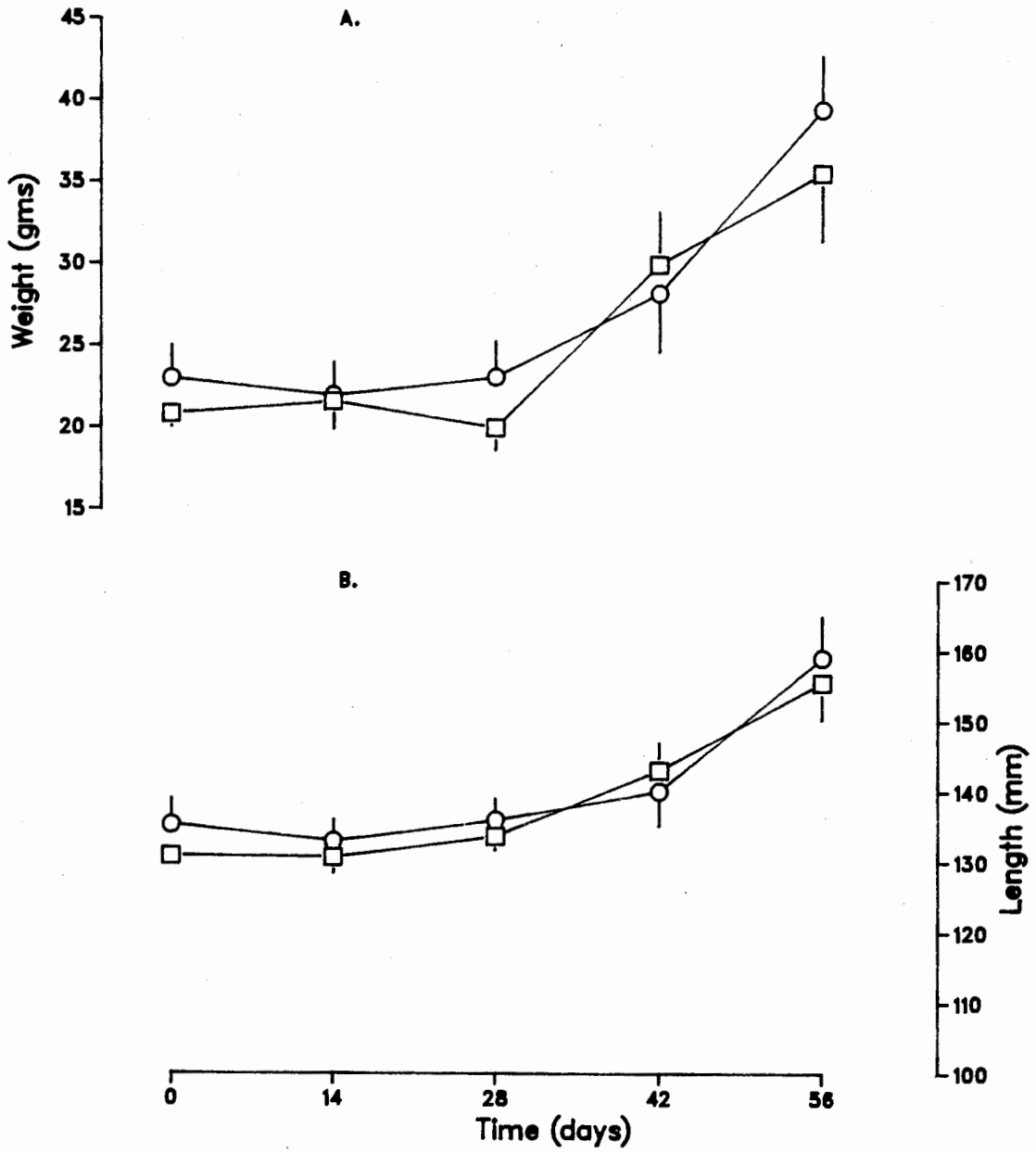
Figure 5. The average growth of coho salmon yearlings (age 1+) (n=8) fed clonidine (□) versus control (○) reported as (A) mean weights (gm) and (B) mean lengths (mm) over 56 days. The vertical lines represent the SEM and were omitted if they overlapped.
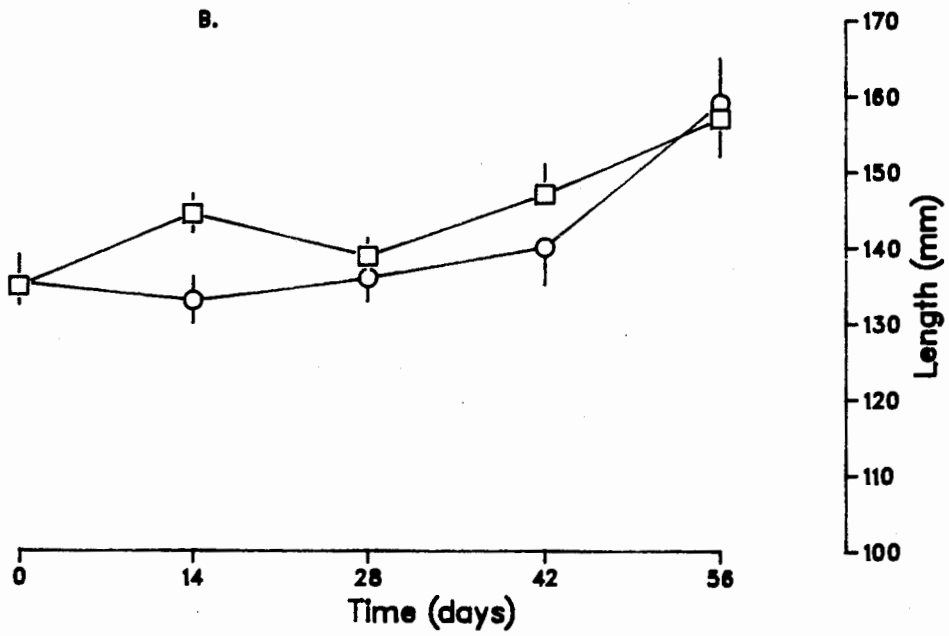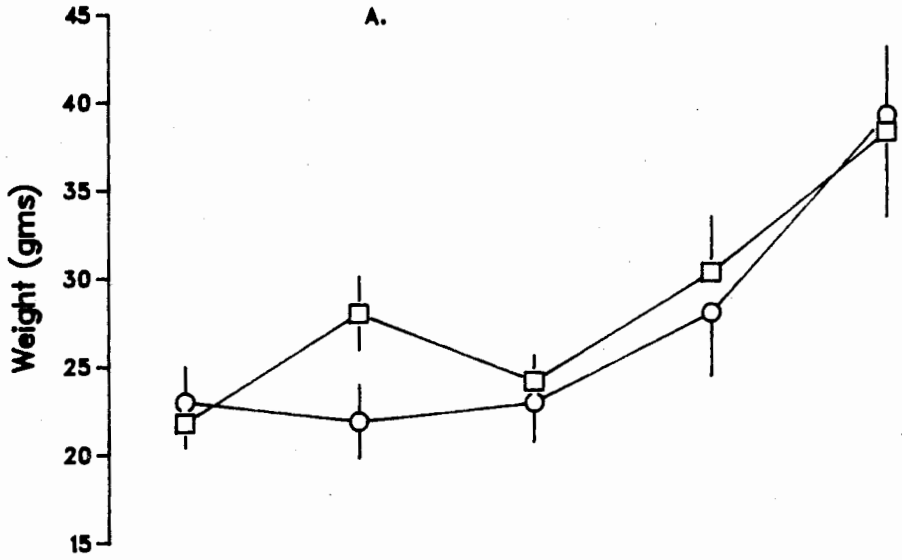
p=0.487) groups.

Results of the BCAA supplemented diet show an increase in plasma GH in coho yearlings (Fig. 6). Plasma GH significantly increased (p=0.010) at day 42 (33.5 ± 7.6 ng/ml) and exceeded the levels found in the controls (9.2 ± 1.0 ng/ml) by 264%. Plasma GH was also significantly elevated (p=0.035) at day 56 (39.0 ± 10.0 ng/ml) and exceeded the levels found in the controls (15.1 ± 1.9 ng/ml) by 158%. Hematocrit levels recorded at the end of the 56 day period did not differ between the treated (33.4 ± 0.8%, n=5) and the controls (36.4 ± 1.6%, n=5).

Food supplemented with AA was not found to elicit any response in the plasma GH of coho yearlings (Fig. 7). Hematocrit levels recorded at the end of the 56 day period did not differ significantly between the treated (34.5 ± 1.5%, n=5) and the controls (36.4 ± 1.6%, n=5).

The GABA supplemented diet did increase plasma GH of coho yearlings (Fig. 8). Plasma GH was significantly increased (p=0.046) at day 28 (55.4 ± 12.2 ng/ml) and exceeded control levels (25.8 ± 7.8 ng/ml) by 115%. The level of plasma GH then dropped in the treated group and by day 42 and 56 (53.7 ± 8.8 ng/ml; 29.9 ± 8.7 ng/ml) did not significantly differ from the controls (40.5 ± 6.6 ng/ml; 33.9 ± 6.0 ng/ml). Hematocrit levels recorded at the end of the 56 day experimental period did not differ significantly between the treated (36.5 ± 0.6%, n=5) and controls (36.4 ± 1.6%, n=5).

Figure 6. The concentration of plasma growth hormone found in coho yearlings (age 1+) fed BCAA (////) (n=10) versus controls ( ) (n=10) over 56 days. Vertical lines represent the SEM and the * represents a significant difference between groups at $p \leq 0.05$.
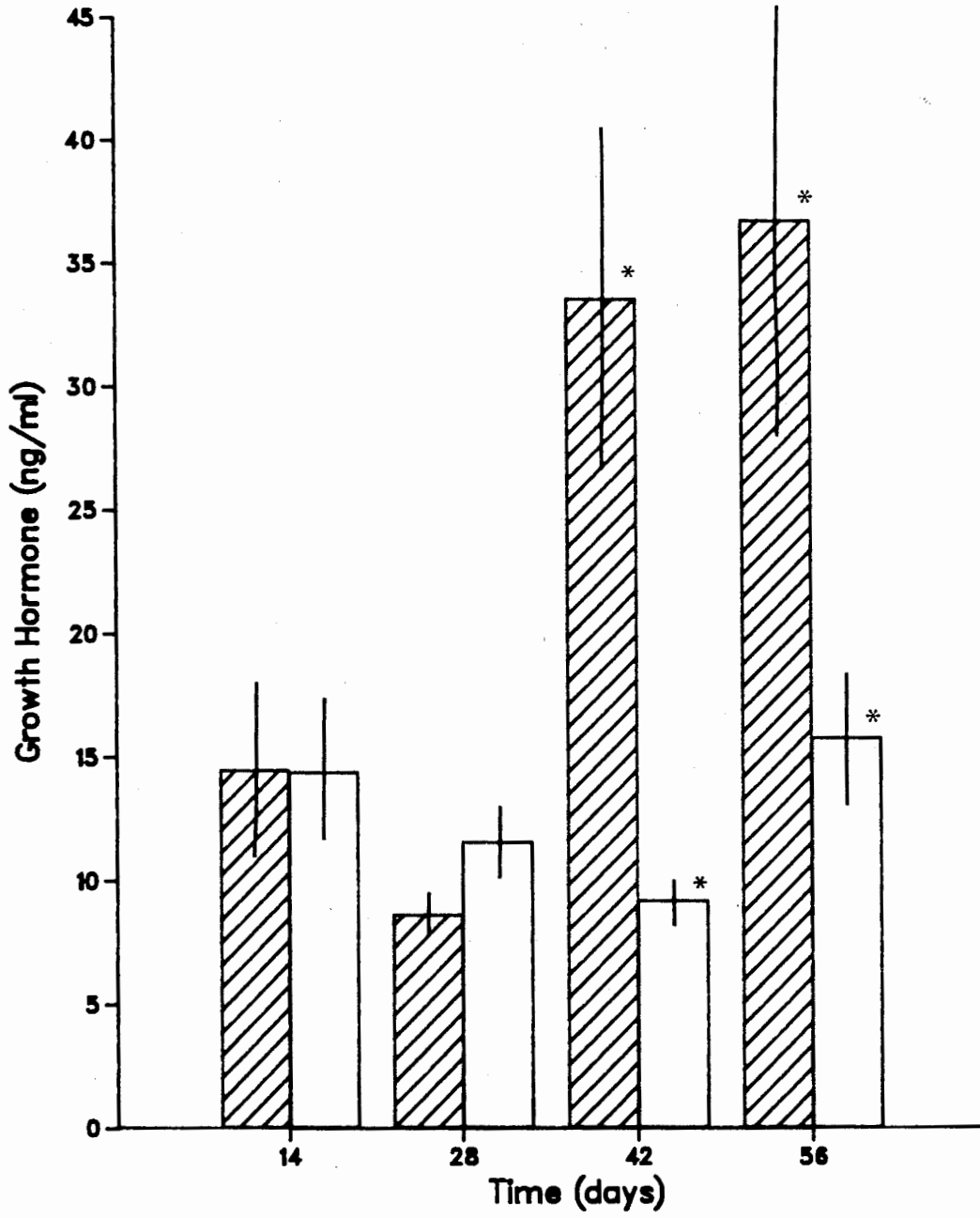
Figure 7. The concentration of plasma growth hormone found in coho yearlings (age 1+) fed AA (〰) (n=8) versus controls ( ) (n=8) over 56 days. Vertical lines represent the SEM and * represents a significant difference between groups at $p \leq 0.05$.
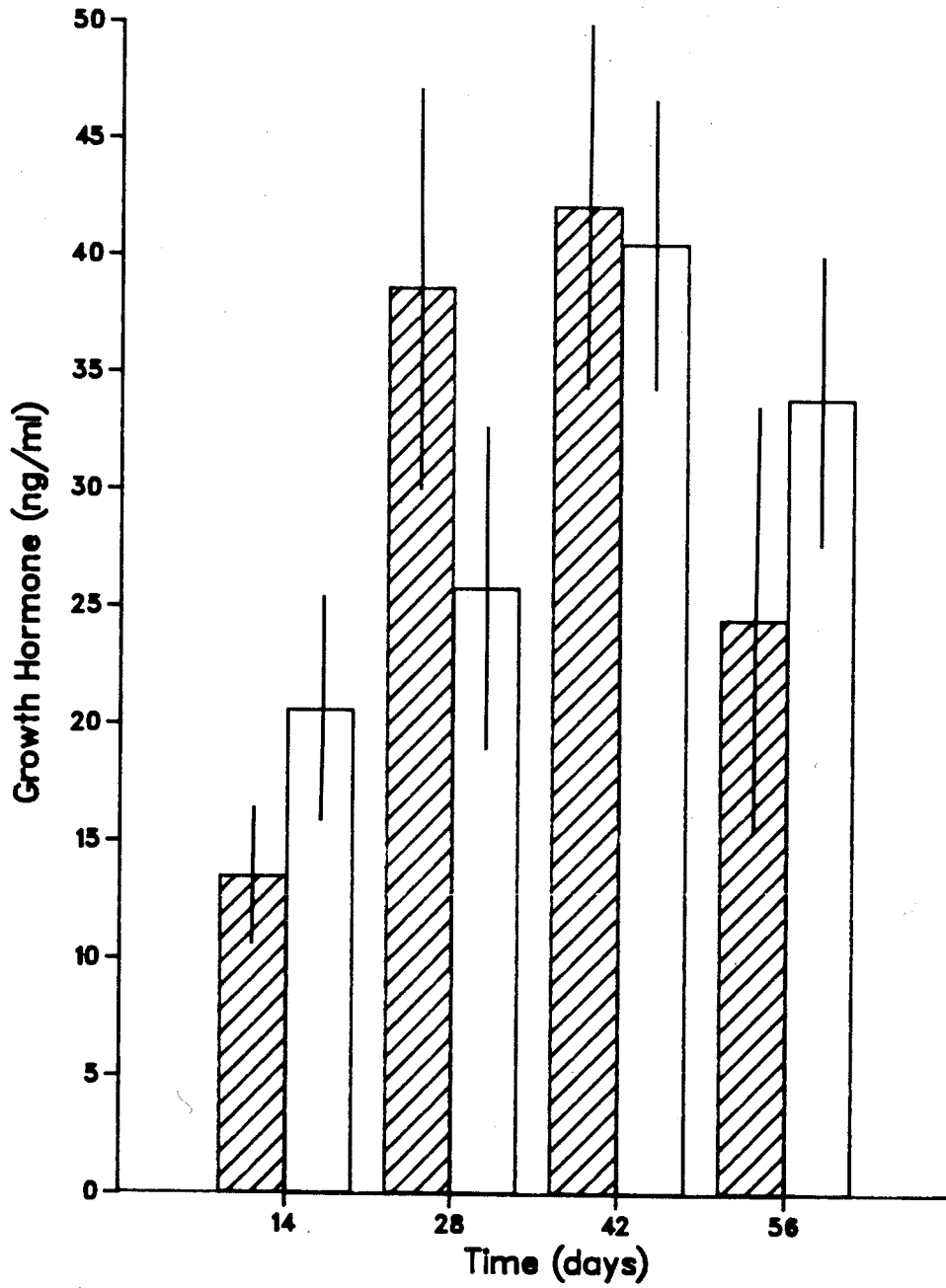
Figure 8. The concentration of plasma growth hormone found in coho yearlings (age 1+) fed GABA (////) (n=8) versus controls ( ) (n=8) over 56 days. Vertical lines represent the SEM and * represents a significant difference between groups at $p \leq 0.05$.
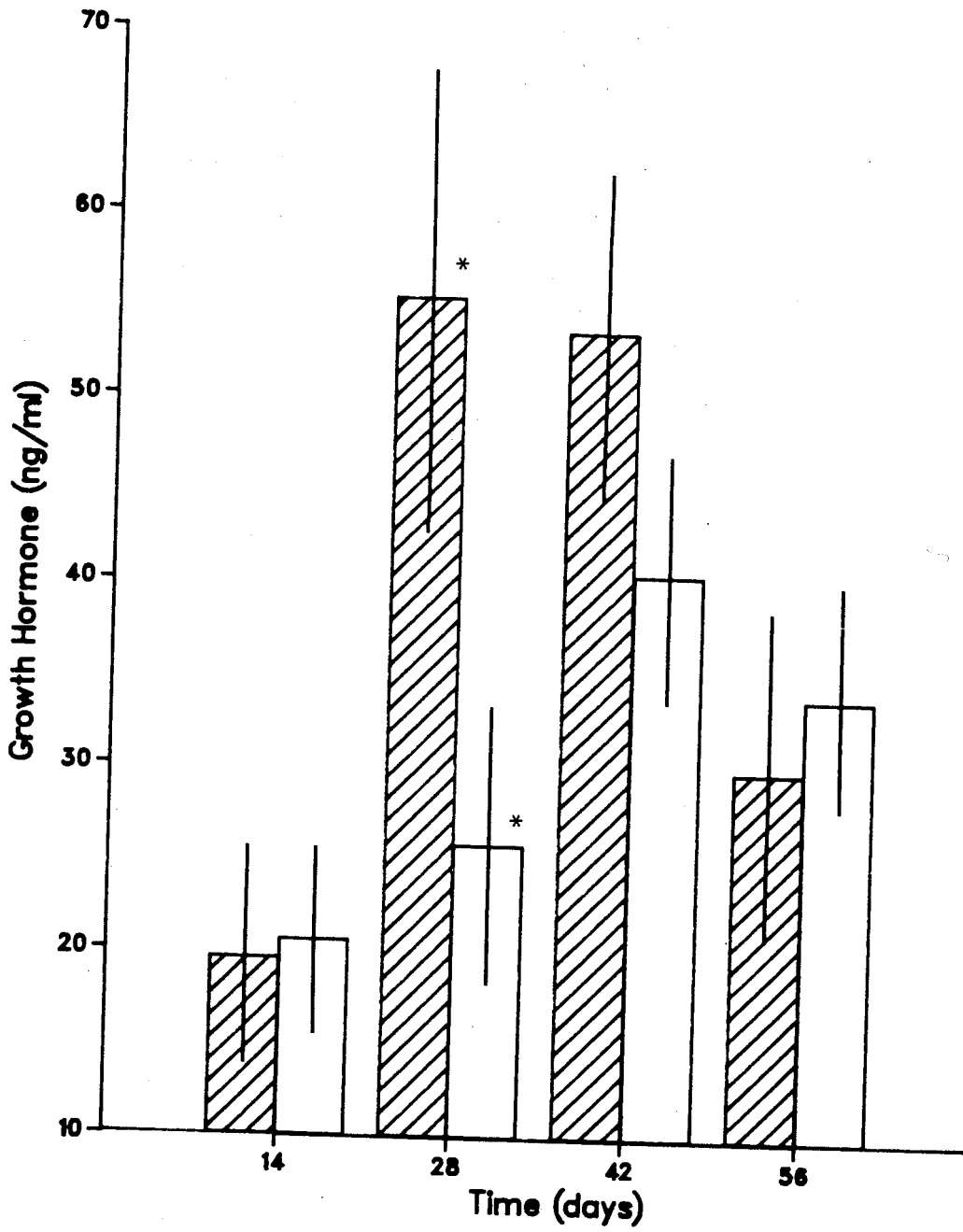
Coho yearlings fed with clonidine supplemented diets, had an immediate response in plasma GH (Fig. 9). Plasma GH was significantly elevated (p=0.037) after 14 days of treatment (48.7 ± 10.9 ng/ml) exceeding the controls (20.6 ± 5.8 ng/ml) by 136%. Levels remained significantly elevated (p=0.023) on day 28 (55.7 ± 7.6 ng/ml) again exceeding the controls (25.8 ± 7.8 ng/ml) by 116%. Following day 28 a decrease in plasma GH was recorded and on days 42 and 56 treated (47.0 ± 10.7 ng/ml, 41.8 ± 10.1 ng/ml) did not differ from the controls (40.5 ± 6.6 ng/ml, 33.9 ± 6.0 ng/ml). Hematocrit levels recorded on day 56 did not differ significantly between the treated (40.8 ± 0.5%, n=5) and the controls (36.4 ± 1.6%, n=5).

Relationships between GH versus weight and GH versus length of individual fish were found to be significant in only 1 of 4 treatment groups.

Fish fed with BCAA, GABA or clonidine supplemented diets did not show any significant correlation between either GH versus weight or GH versus length at any time during the experimental period.

Fish fed with AA supplemented diet showed a correlation between GH versus weight (Fig. 10) and GH versus length (Fig. 11). Significant correlations between GH versus weight were found on day 42 (F=7.134, p=0.037, logY=3.34-1.27logX, r2=0.540) and day 56 (F=8.923, p=0.031, logY=6.07-3.14logX, r2=0.641). Length showed a significant correlation with GH only on day 56 (F=8.476, p=0.033, logY=21.0-9.10logX,

Figure 9. The concentration of plasma growth hormone found in coho yearlings (age 1+) fed clonidine (///) (n=8) versus controls ( ) (n=8) over 56 days. Vertical lines represent the SEM and * represents a significant difference between groups at p≤0.05.
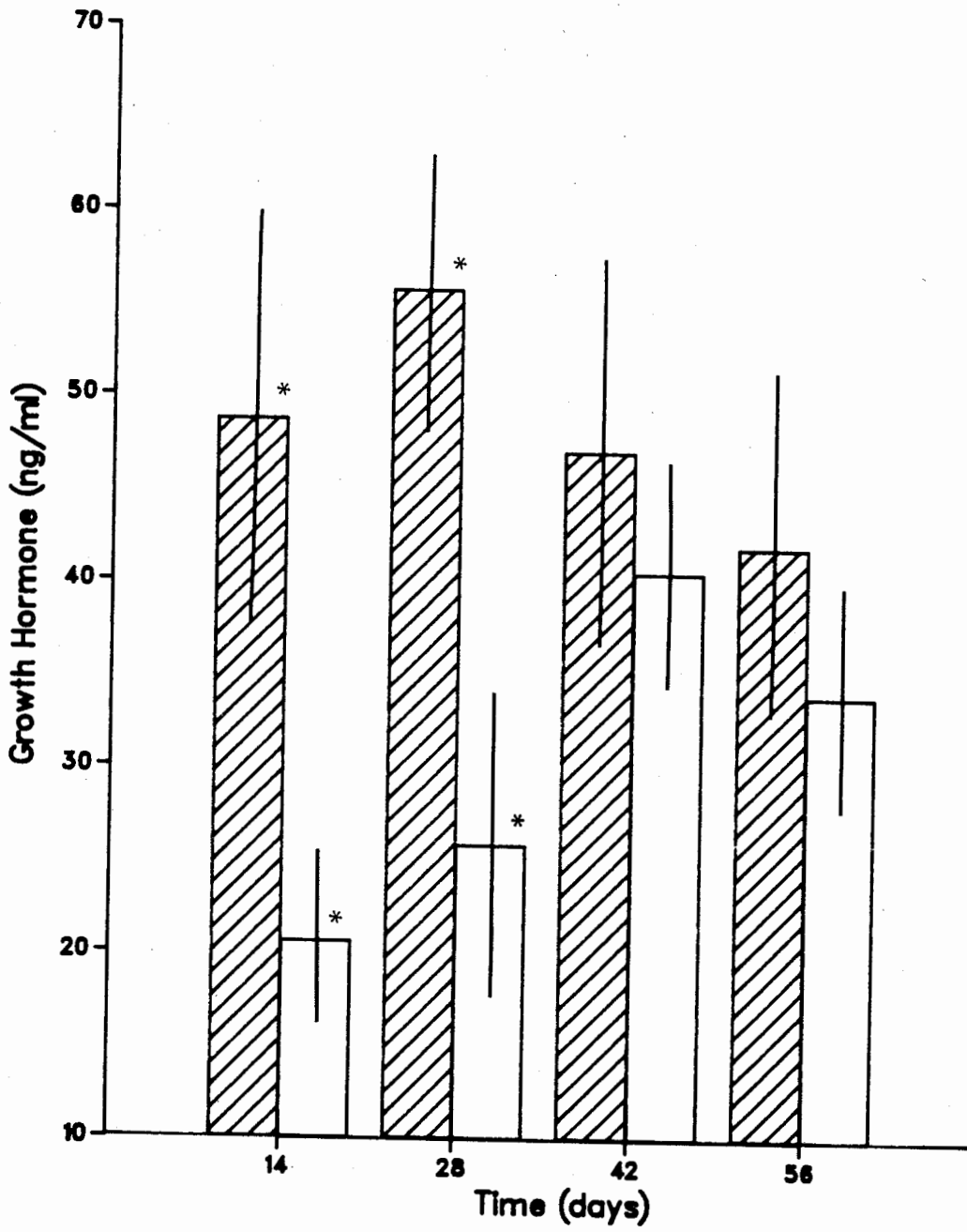
Figure 10. The model II linear regressions between GH and weight in coho yearlings (age 1+; n=8) fed AA. (A) after 2 weeks of treatment (p=0.521); (B) after 4 weeks of treatment (p=0.072); (C) after 6 weeks of treatment (logY=3.34-1.27logX; r2=0.540; p=0.037); (D) after 8 weeks of treatment (logY=6.07-3.14logX;r2=0.641; p=0.031).Each point represents one fish and lines are plotted if p≤0.05.
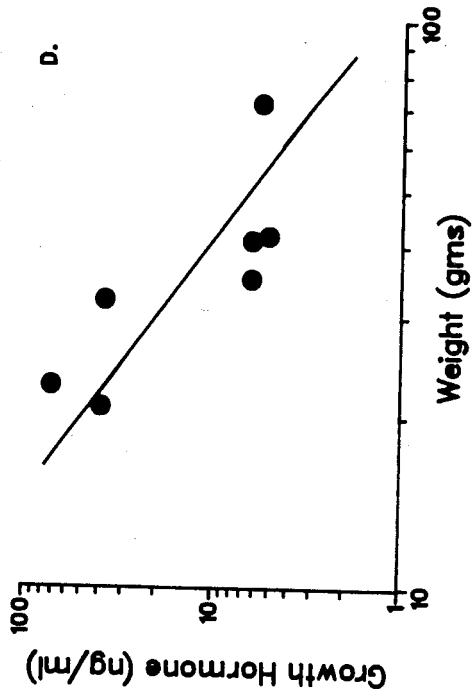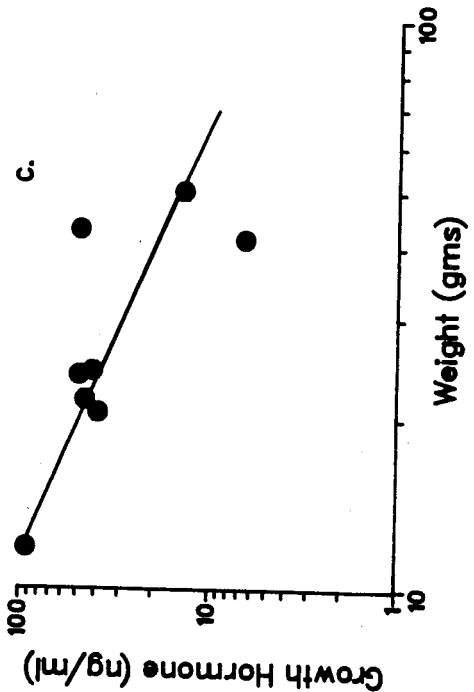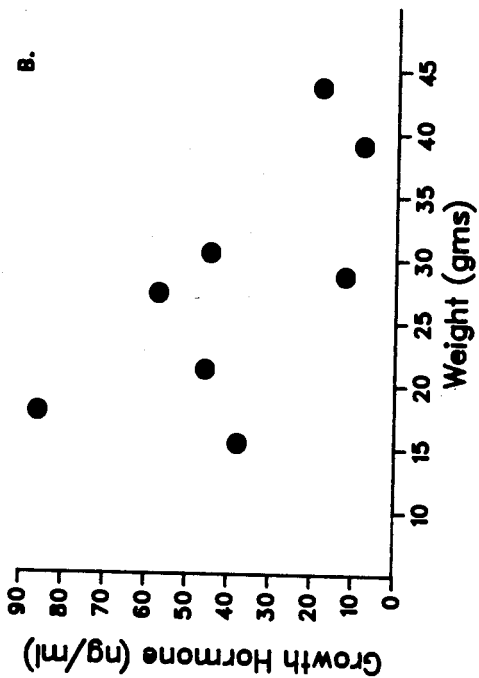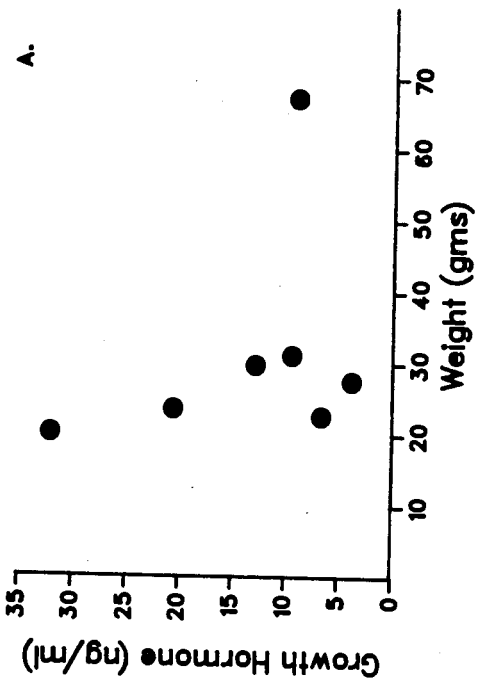
Figure 11. The model II linear regressions between GH and length in coho yearlings (age 1+; n=8) fed AA. (A) after 2 weeks of treatment (p=0.795); (B) after 4 weeks of treatment (p=0.086); (C) after 6 weeks of treatment (p=0.118); (D) after 8 weeks of treatment (logY=21.0-9.10logX; r2=0.630; p=0.033). Each point represents one fish and lines are plotted if $p \leq 0.05$.
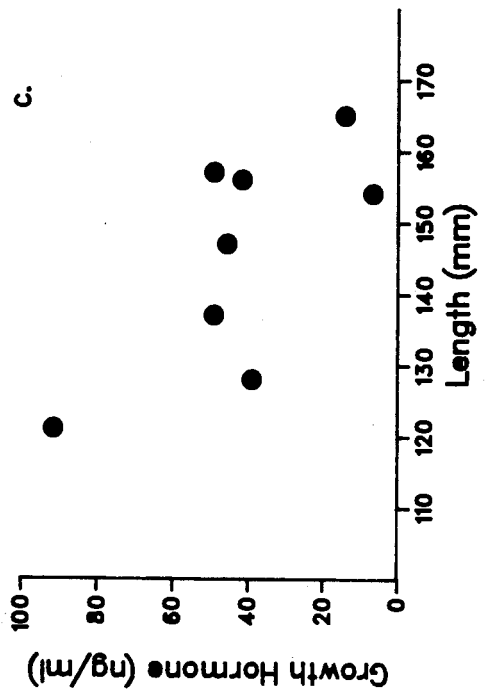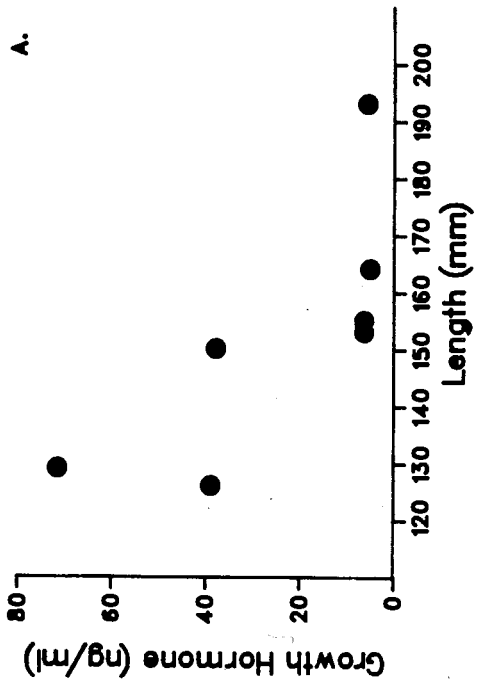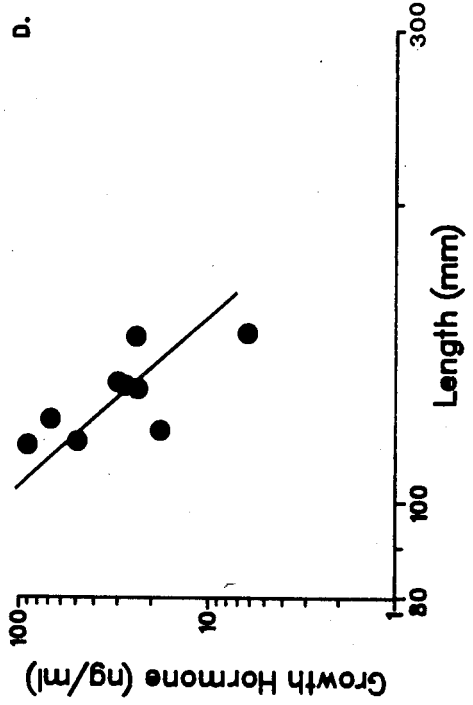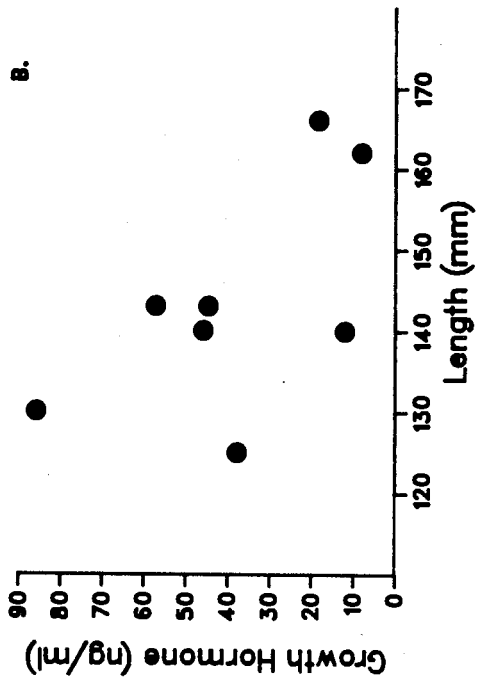
r2=0.63).

. The rate of BCAA leaching from the feed into water, showed a steady increase in all three BCAA occurring within the first 30 minutes. After 30 minutes the amount of BCAA found in the water started to drop. The percentage of the total valine found in the water after 5, 15, 30 and 60 minutes was 19.7%, 41.8%, 48.3% and 46.2% respectively. The percentage of the total isoleucine is the water after 5, 15, 30 and 60 minutes was 13.8%, 19.9%, 33.3% and 33.0% respectively. The percentage of total leucine found in the water after 5, 15, 30 and 60 minutes was 22.2%, 69.4%, 74.4% and 72.9% respectively.

Plasma concentrations of the BCAA in treated fish were also significantly elevated over control fish by day 56. Plasma valine in treated fish (421.5 ± 26.4 nmol/ml) exceeded the controls (319.0 ± 15.7 nmol/ml) by 32% (p=0.010, n=5). Plasma isoleucine in treated fish (169.0 ± 13.1 nmol/ml) exceeded the controls (75.4 ± 6.3 nmol/ml) by 124% (p<0.001, n=5). Plasma leucine in treated fish (274 ± 18.3 nmol/ml) exceeded the controls (156.0 ± 11.8 nmol/ml) by 76% (p<0.001, n=5).

The results from the amino acid analysis of Ewos fishfeed are illustrated in table V. Only 9 of the 10 essential amino acids could be measured using the HPLC technique described earlier. The amino acids of importance to this study, namely: aspartic acid, arginine, valine, isoleucine and leucine contributed 2.9%, 2.6%, 2.7%, 2.0%

Table V. Amino acid composition of a commercial brandname
fish feed. Values are expressed as a percentage of 100 mg of
dry diet. The * highlights 9 of the 10 essential amino
acids.

Amino acid	% dry diet	Amino acid	% dry diet
ASP	2.9	*ARG	2.6
GLU	5.9	TYR	1.5
SER	2.2	*VAL	2.7
GLY	2.8	*MET	1.4
*HIS	2.1	*ILE	2.0
*THR	2.5	*LEU	3.8
ALA	3.0	*PHE	2.3
PRO	2.2	*LYS	3.0

and 3.8% per 100 mg of dry diet respectively.

3.3 Experiment 3: Treated Coho Fry

Coho salmon fry fed supplemented diets containing BCAA (Fig. 12), AA (Fig. 13), GABA (Fig. 14) and clonidine (Fig. 15) all showed significant increases in growth over controls.

In the treatment group fed with BCAA, a significant increase in both weight (F=33.346, p<0.001) and length (F=28.224, p<0.001) was found at the end of 70 days (Fig. 12). There was a significant interactive effect of treatment and time on weight (F=2.977, p=0.013) and length (F=3.407, p=0.005). Final weight of the treated fish (2.28 ± 0.14 g, n=20) exceeded the controls (1.50 ± 0.08 g, n=20) by 52%. The final length of the treated fish (56.8 ± 1.1 mm) exceeded the controls (50.3 ± 0.8 mm) by 13%.

Fish fed with AA supplemented diet also significantly increased in both weight (F=6.369, p=0.012) and length (F=8.206, p=0.005) at the end of 70 days (Fig. 13). No significant interaction between treatment and time was found on either weight (F=1.711, p=0.133) or length (F=1.747, p=0.125). Final weight of the treated fish (1.97 ± 0.15 g, n=20) exceeded the controls (1.50 ± 0.08 g, n=20) by 31%. Length of the treated fish (54.4 ± 1.2 g, n=20) exceeded the controls (50.3 ± 0.8 mm) by 8%.

Fish fed with GABA supplemented diet were also significantly larger in weight (F=10.407, p=0.001) and

Figure 12. The average growth of coho salmon fry (age 0+)
(n=20) fed BCAA (▢) versus controls (○) reported as (A)
mean weights (gm) and (B) mean lengths (mm) over 56 days.
The vertical lines represent the SEM and were omitted if
they overlapped. The * represents a significant difference
between groups at p≤0.05.

45a
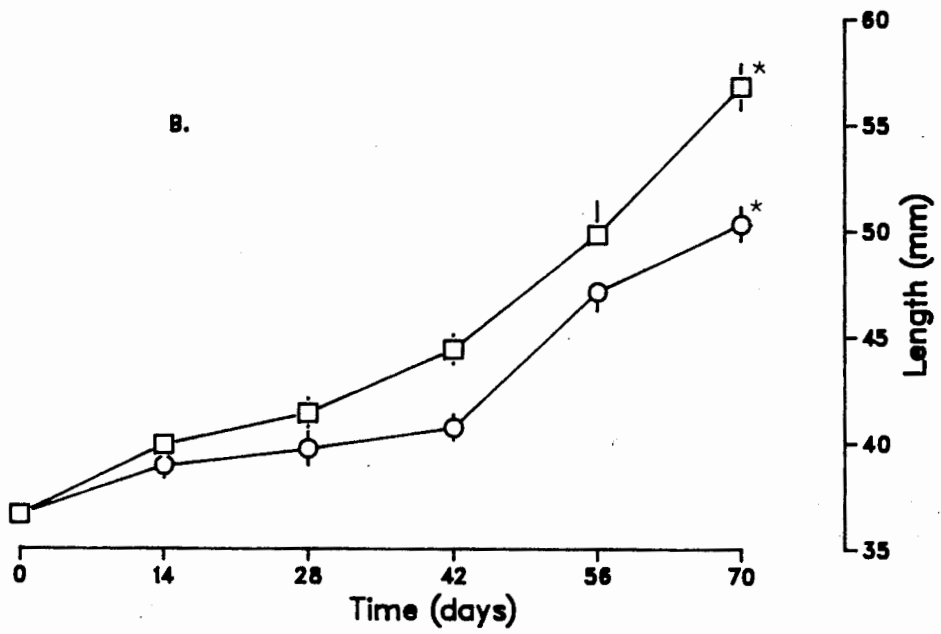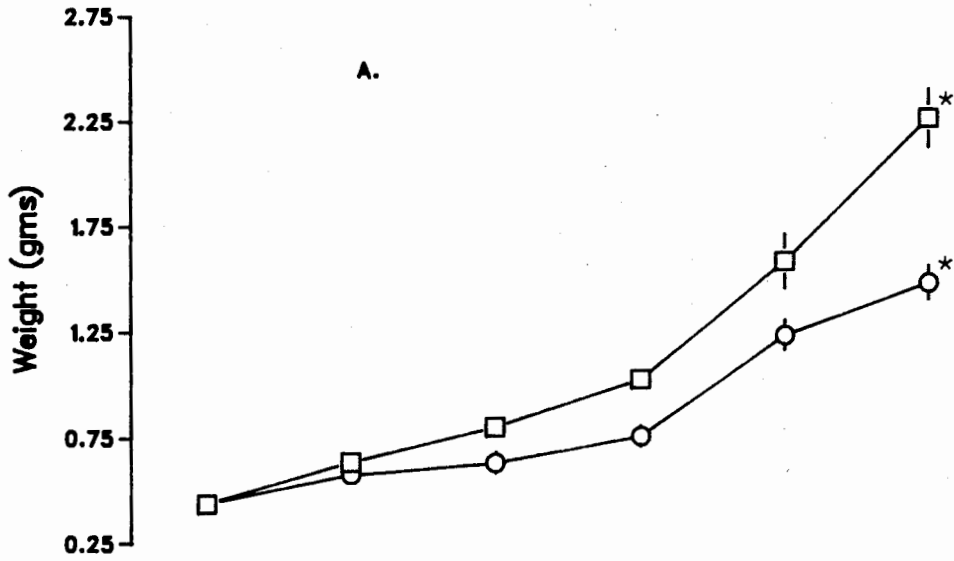
Figure 13. The average growth of coho salmon fry (age 0+) (n=20) fed AA (□) versus controls (○) reported as (A) mean weights (gm) and (B) mean lengths(mm) over 56 days. The vertical lines represent the SEM and were omitted if they overlapped. The * represents a significant difference between groups at $p \leq 0.05$.
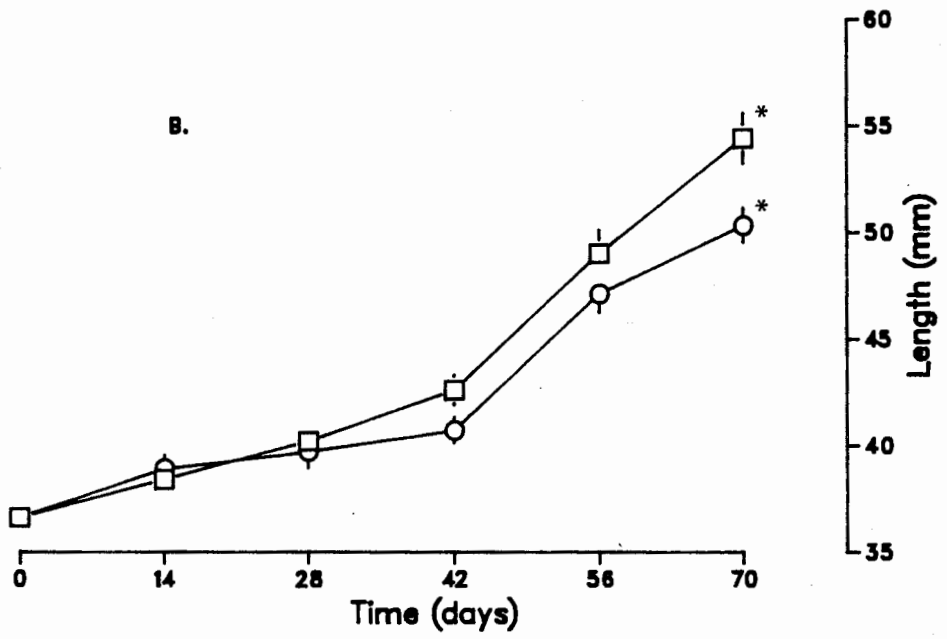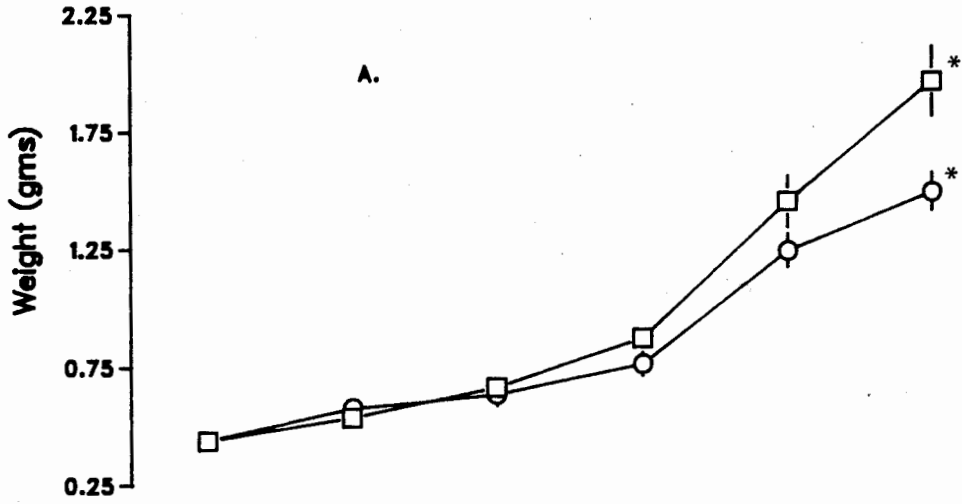
Figure 14. The average growth of coho salmon fry (age 0+)
(n=20) fed GABA (☐) versus controls (○) reported as (A)
mean weights (gm) and (B) mean lengths (mm) over 56 days.
The vertical lines represent the SEM and were omitted if
they overlapped. The * represents a significant difference
between groups at p≤0.05.
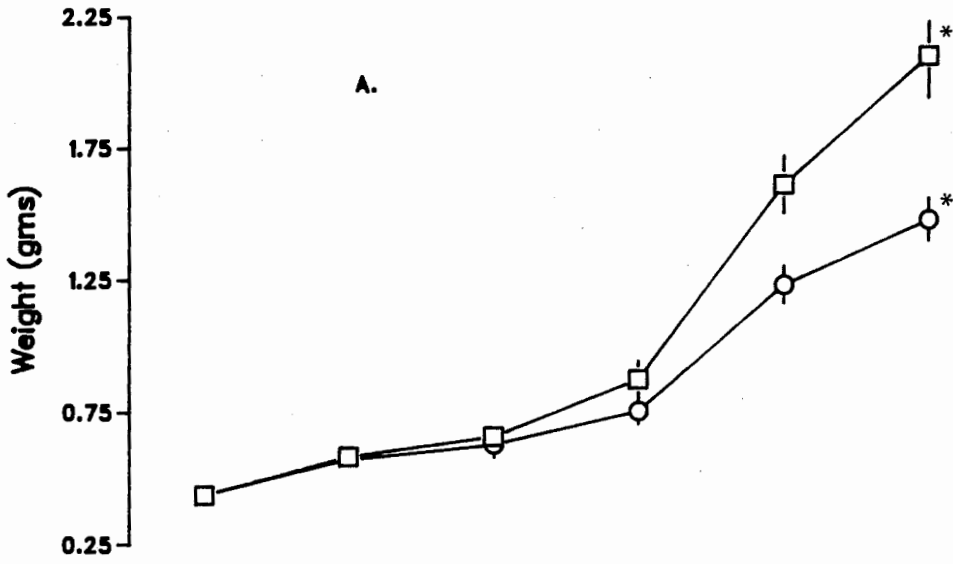
Figure 15. The average growth of coho salmon fry (age 0+) (n=20) fed clonidine (□) versus controls (O) reported as (A) mean weights (gm) and (B) mean lengths (mm) over 56 days . The vertical lines represent the SEM and were omitted if they overlapped. The * represents a significant difference between groups at $p \leq 0.05$.
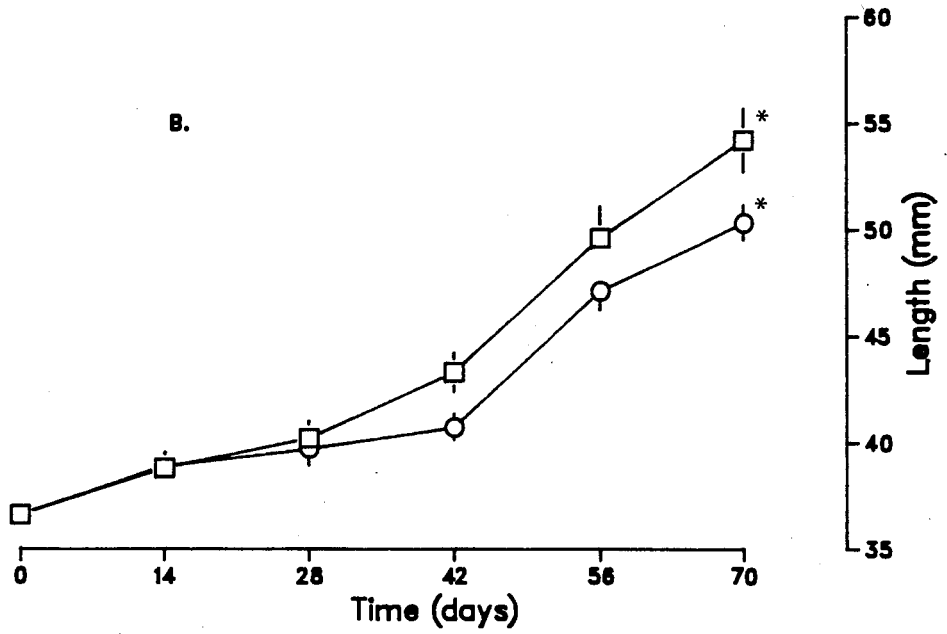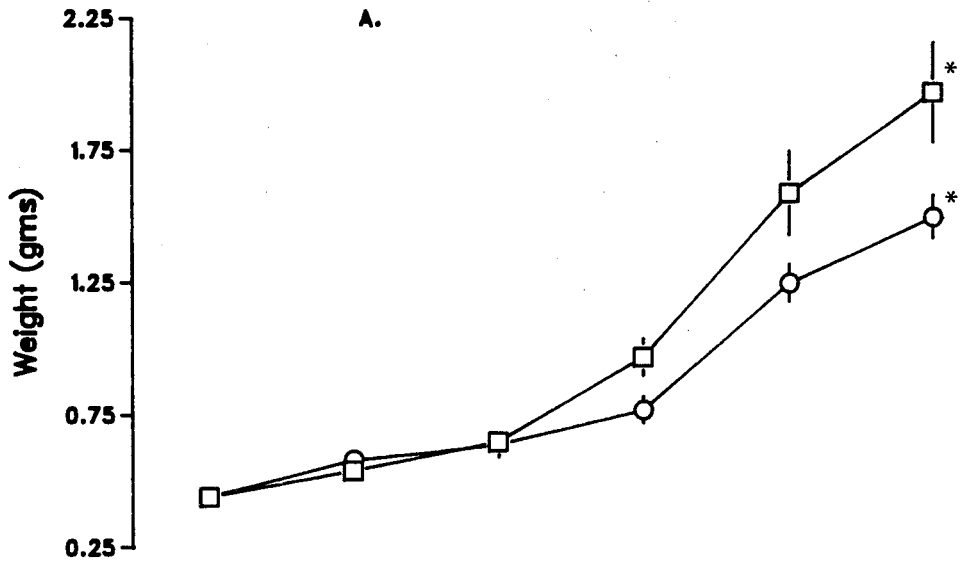
length (F=17.367, p<0.001) at the end of 70 day (Fig.14). No
significant interactive effect between treatment and time
was found on weight (F=2.074, p=0.069), while a significant
effect was found on length (F=2.585, p=0.027). Final weight
of the treated fish (2.12 ± 0.16 g, n=20) exceeded the
controls (1.50 ± 0.08 g, n=20) by 41%. Length of the treated
fish (56.5 ± 1.4 mm) exceeded the controls (50.3 ± 0.8 mm)
by 12%.

Clonidine treated fish were also significantly larger
in weight (F=4.428, p=0.036) and length (F=5.756, p=0.017)
after 70 days (Fig. 15). No significant interactive effects
between treatment and time were found on either weight
(F=1.671, p=0.143) or length (F=1.321, p=0.236). Final
weight of the treated fish (1.99 ± 0.19 g, n=20) exceeded
the controls (1.50 ± 0.08 g, n=20) by 33% and length of the
treated fish (54.2 ± 1.5 mm) exceeded the controls (50.3 ±
0.8 mm) by 8%.

Results of the weight and length comparisons between
the control fish fed commercial diet with Canola oil versus
control fish fed only commercial diet showed no significant
differences (p≥0.05, Fig. 16).

Mortalities over the course of the 70 day experiment
for the control, BCAA, AA, GABA and clonidine treated fish
were 4.2%, 2.0%, 2.3%, 5.3% and 7.8% resectively.

3.4 Experiment 4: BCAA Concentrations

Coho fry fed with three concentrations of BCAA (1.3

Figure 16 The average growth of coho salmon fry (age 0+; n=20) fed canola oil (O) versus controls (□) reported as (A) mean weights (gm) and (B) mean lengths (mm) over 70 days. The vertical lines represent the SEM and were omitted if they overlapped.
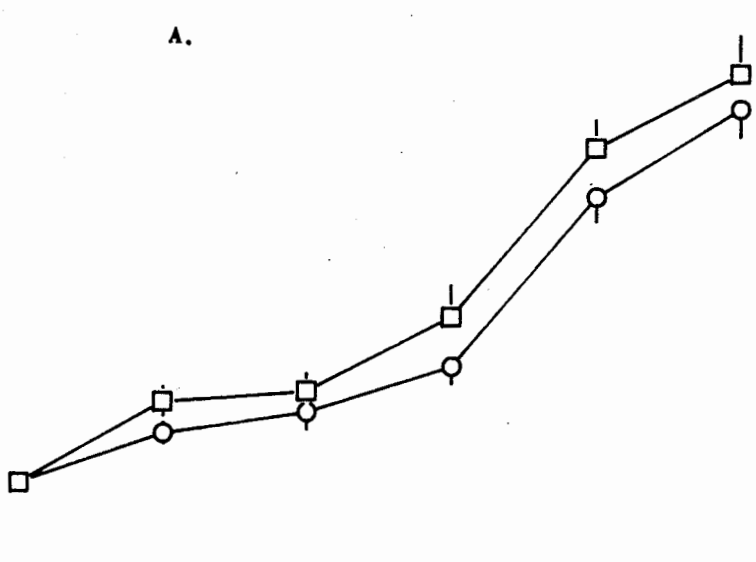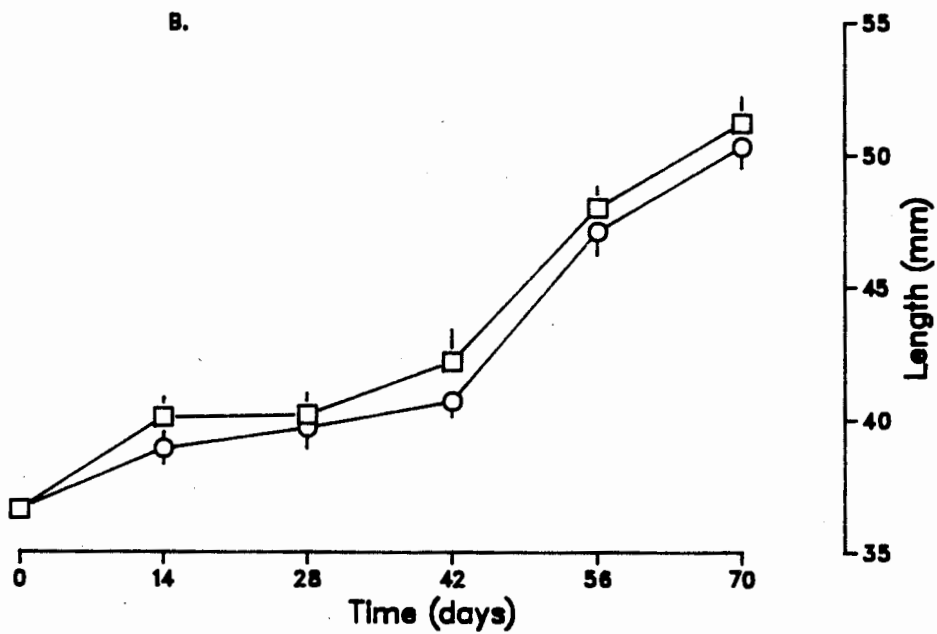
mg/g, 3.8 mg/g, 11.4 mg/g) at two different ratios (1:1:1, 2:2:1) showed no significant differences in either weight (Fig. 17, 1:1:1; F=0.097, p=0.908; 2:2:1; F=2.202, p=0.113) or length (Fig. 18, 1:1:1; F=0.535, p=0.586; 2:2:1; F=1.456, p=0.235) at the end of 56 days. In the groups receiving BCAA at a ratio of 1:1:1 there were no significant interactive effects between concentration and time on either weight (F=0.509, p=0.849) or length (F=0.403, p=0.918). Similarly, in the group receiving BCAA at a ratio of 2:2:1 no significant interactive effects between concentration and time were found on either weight (F=0.263, p=0.977) or length (F=0.226, p=0.986).

Comparisons between the different ratios of BCAA at the same total concentration did not reveal any significant changes in growth. Figure 19 shows the changes in weight F=0.593, p=0.442) and length (F=0.963, p=0.328) for fish fed with 1.3 mg/g BCAA at the ratios of 1:1:1 and 2:2:1 (val:leu:ile). Figure 20 illustrates the average weight (F=2.137, p=0.146) and length (F=1.501, p=0.222) for fish fed with 3.8 mg/g at the ratios of 1:1:1 and 2:2:1. Figure 21 illustrates the weight (F=1.692, p=0.195) and length (F=2.018, p=0.157) for fish fed with 11.4 mg/g BCAA at 1:1:1 and 2:2:1. There was no significant interaction between concentration and time on weight in the groups fed either 1.3 mg/g (F=0.623, p=0.647), 3.8 mg/g (F=0.130, p=0.971) or 11.4 mg/g (F=0.191, p=0.943). Similarly, no significant interaction between concentration and time on length was

found in groups fed either 1.3 mg/g (F=0.722, p=0.578), 3.8 mg/g (F=0.110, p=0.979) or 11.4 mg/g (F=0.177, p=0.950).

Figure 17. The average weight of coho salmon fry (age 0+; n=20) fed BCAA in concentrations of 1.3 mg/g body weight (□), 3.8 mg/g body weight (●) and 11.4 mg/g body weight (○) where (A) is at a ratio of 1:1:1 (val:leu:ile) and (B) is at a ratio of 2:2:1 (val:leu:ile).

52b

53a
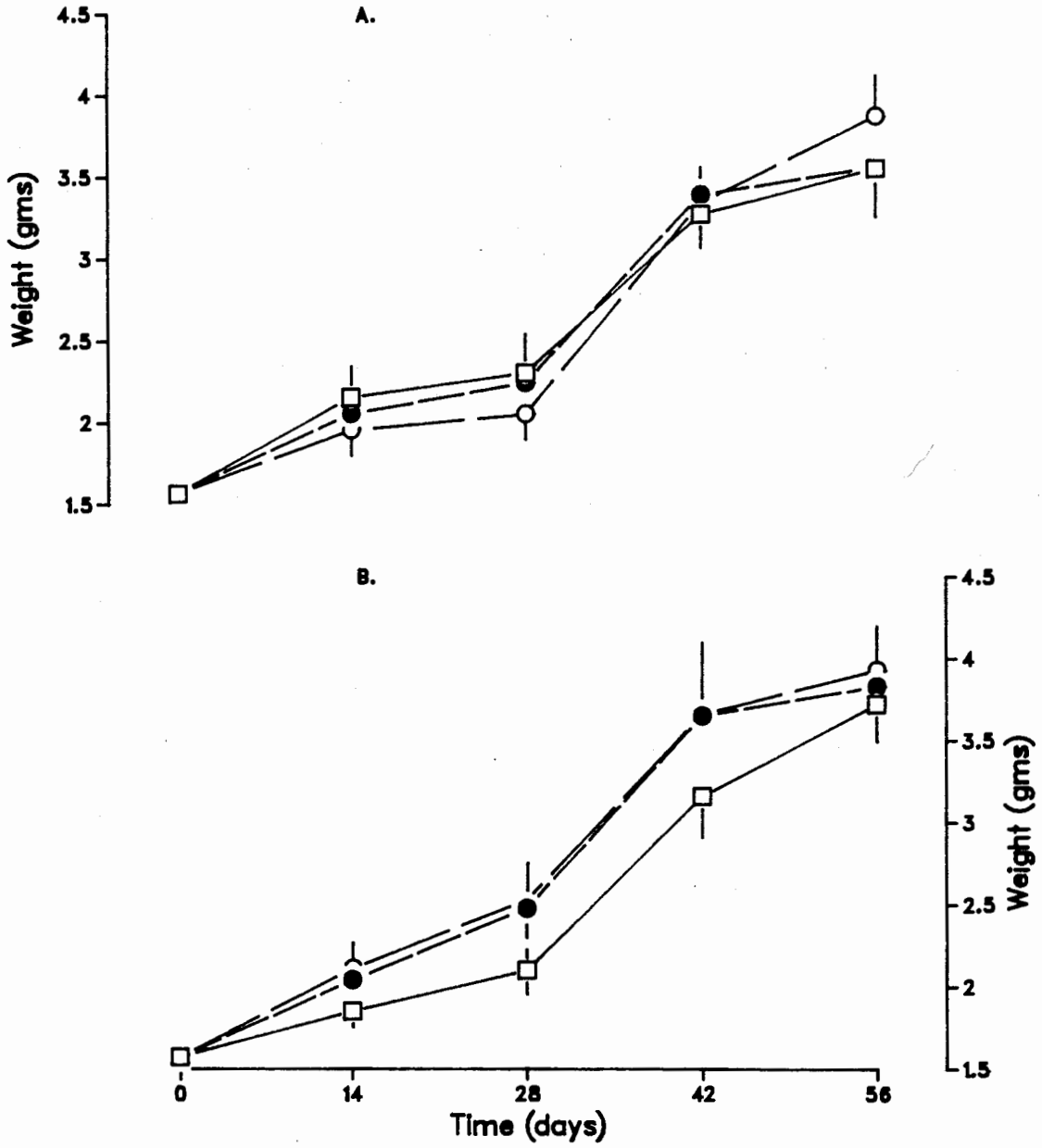
Figure 18. The average length of coho salmon fry (age 0+; n=20) fed BCAA in concentrations of 1.3 mg/g body weight (□), 3.8 mg/g body weight (●) and 11.4 mg/g body weight (○) where (A) is at a ratio of 1:1:1 (val:leu:ile) and (B) is at a ratio of 2:2:1 (val:leu:ile).
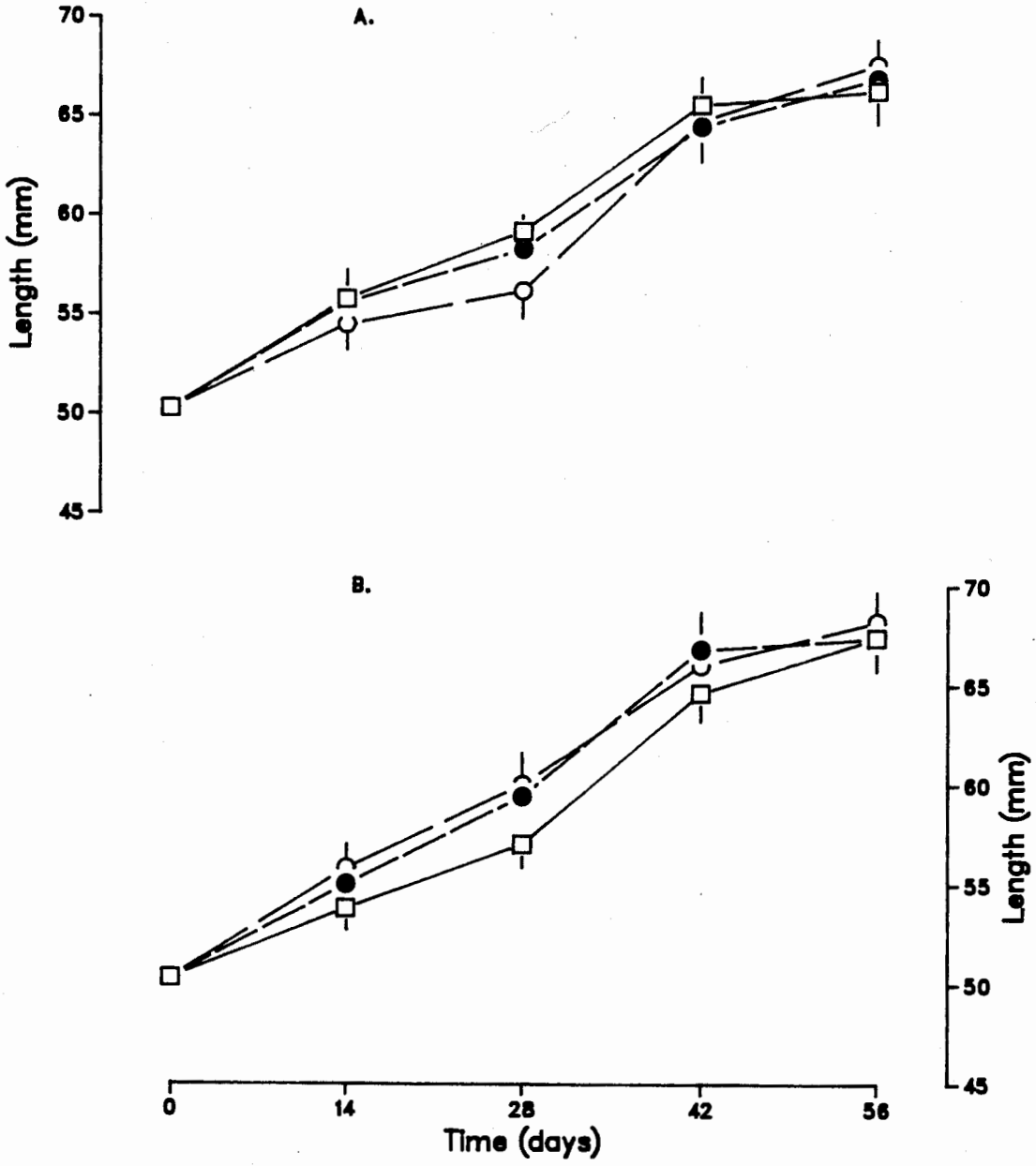
Figure 19. The average growth of coho salmon fry (age 0+; n=20) fed 1.3 mg/g body weight BCAA in the ratio of 1:1:1 (□) and 2:2:1 (○) (val:leu:ile) where (A) is the mean weights and (B) the mean lengths over 56 days. The vertical lines represent the SEM.and were omitted if they overlapped.
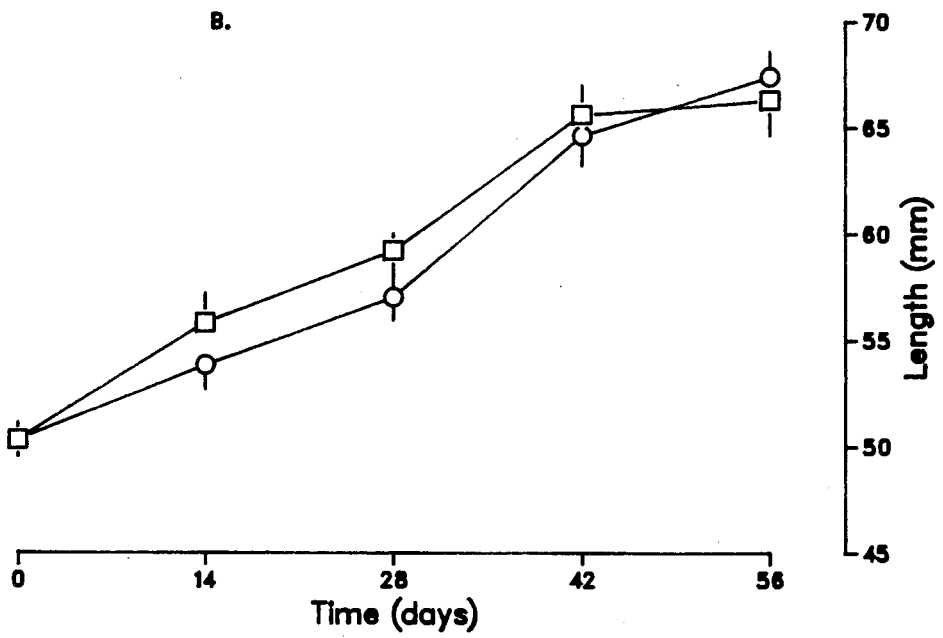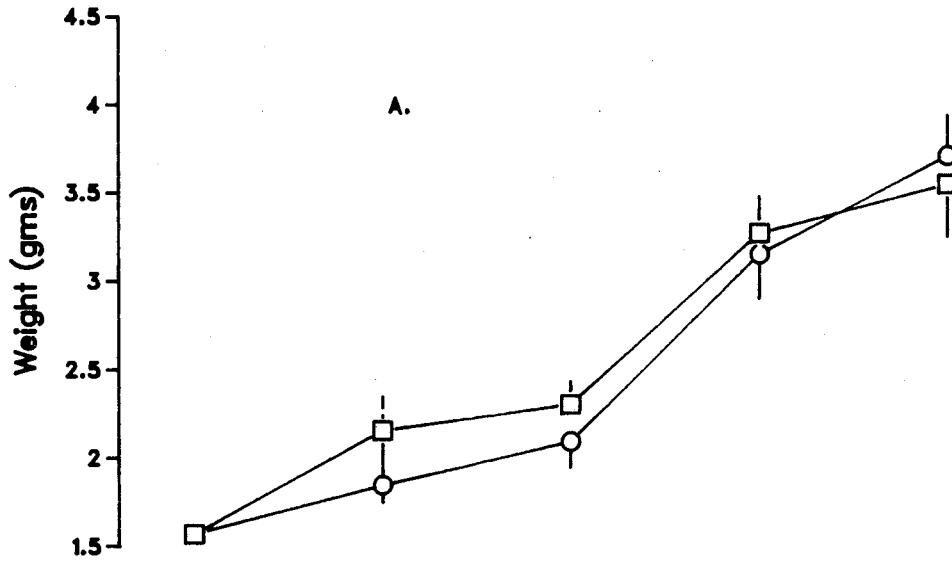
Figure 20. The average growth of coho salmon fry (age 0+; n=20) fed 3.8mg/g body weight BCAA in the ratio of 1:1:1 (□) and 2:2:1 (○) (val:leu:ile) where (A) is the mean weights and (B) the mean lengths over 56 days. The vertical lines represent the SEM and were omitted of they overlapped.

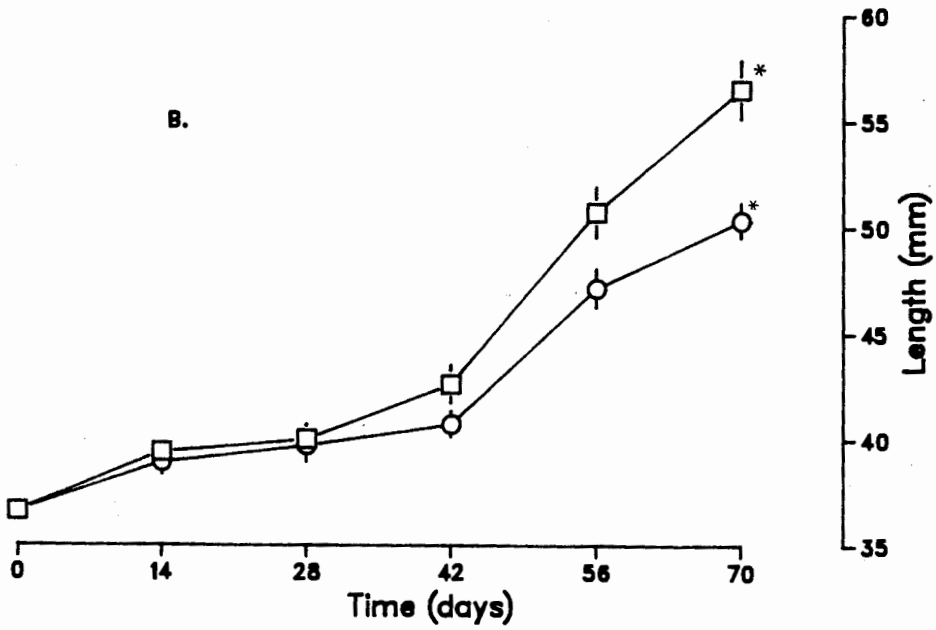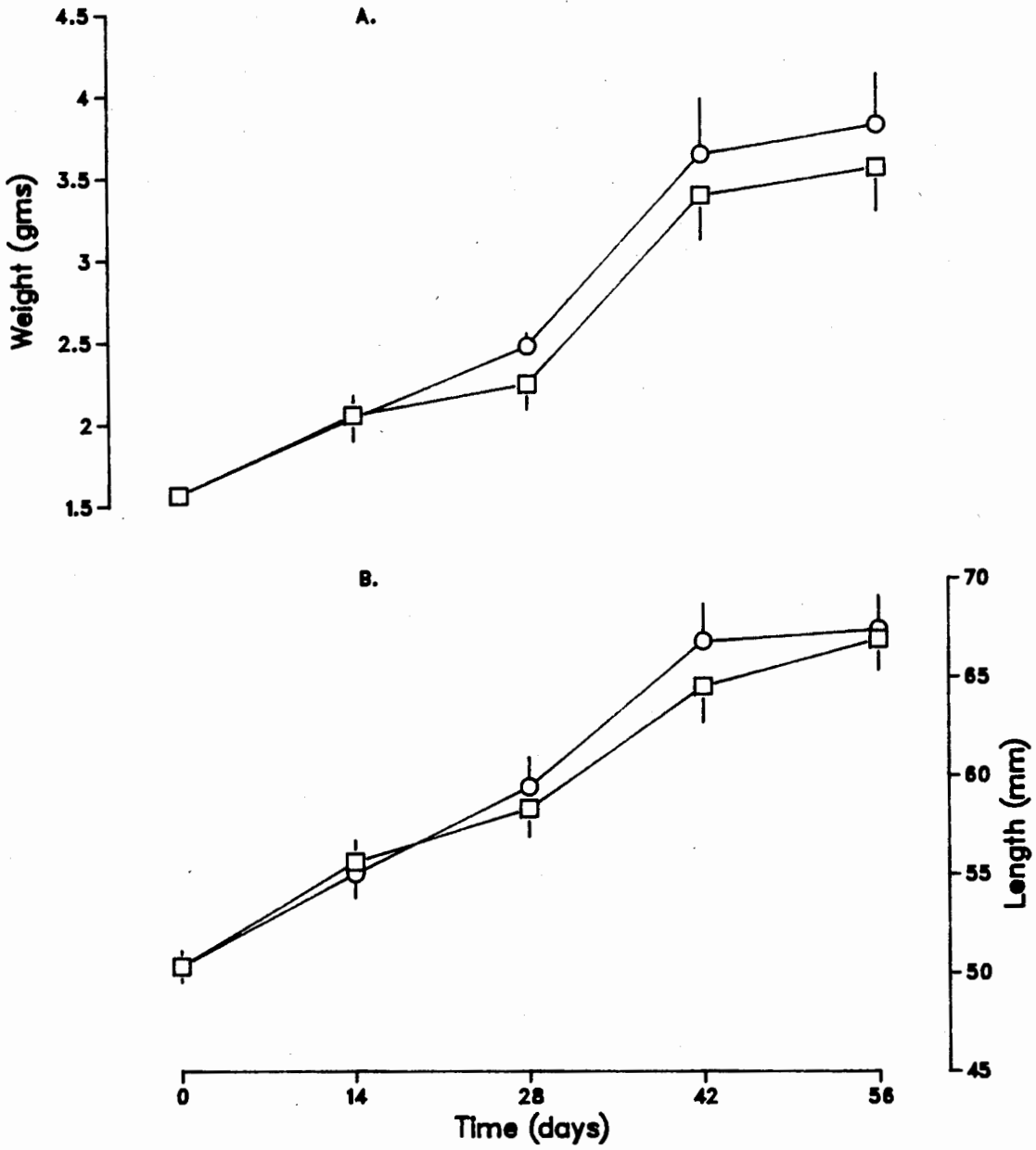**Figure 21.** The average growth of coho salmon fry (age 0+;
n=20) fed 11.4 mg/g body weight BCAA in the ratio 1:1:1 (□)
and 2:2:1 (○) (val:leu:ile) where (A) is the mean weights
and (B) the mean lengths over 56 days. The vertical lines
represent the SEM and were omitted if they overlapped.
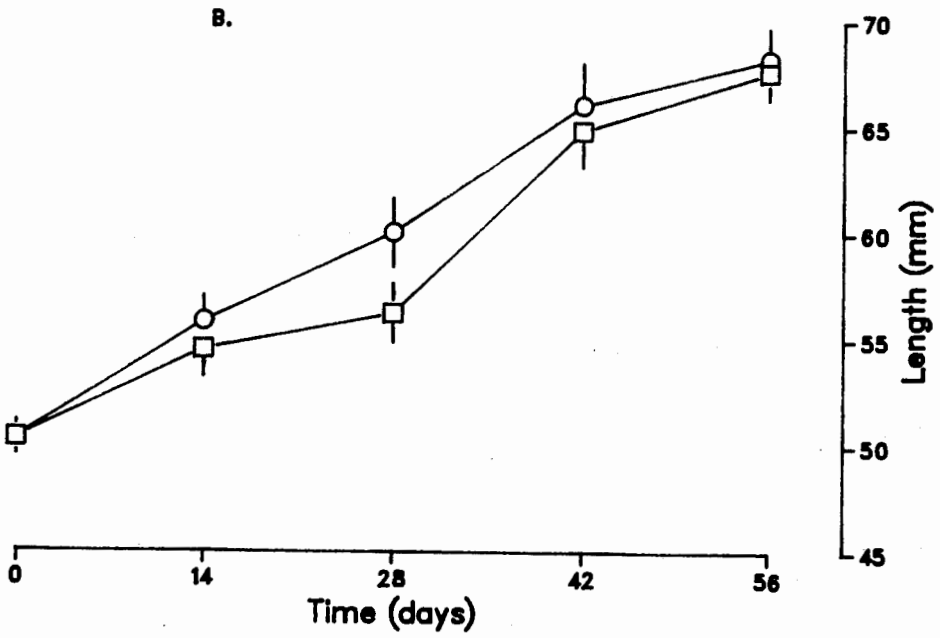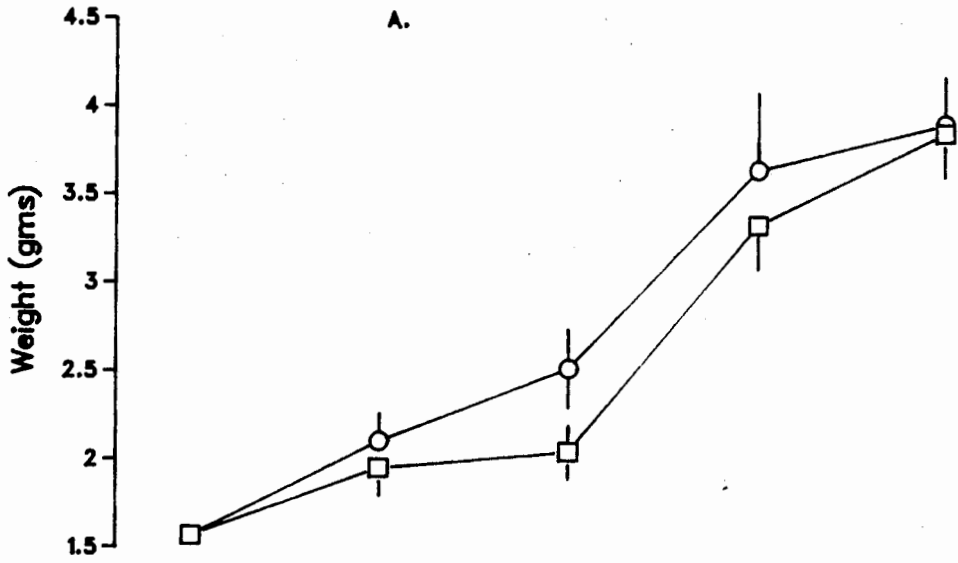
4.0 DISCUSSION

4.1 Branched Chain Amino Acids (BCAA)

The results from the injections of rainbow trout with BCAA show plasma GH was increased over controls (Table I). In fish injected with BCAA, plasma GH was significantly higher than controls 90 minutes after the injections. A similar response in plasma GH has also been described in other vertebrates (Davis, 1972; Stewart et al., 1984).

Davis (1972), infused the BCAA leucine (0.2 g/kg) into sheep, causing a significant increase in plasma GH. The mechanism proposed for this response was that leucine worked at the level of the hypothalamus, stimulating the release of GRF. In baboons, increasing plasma levels of BCAA's by infusion altered the rhythm of GH secretion (Stewart et al., 1984). Stewart et al. (1984) noted that the BCAA's caused an overall increase in the amplitude of the GH peak (205%) and a total GH increase of approximately 4% over 12 hours. Their results conflict somewhat with those presented in table I in that no short term effects were observed as opposed to those observed for rainbow trout. This question of acute (short term) versus chronic (long term) is also contradictory in other studies. Bratusch-Marrian and Waldhausl (1979) found valine did not acutely alter GH release in man, whereas leucine did (1 Hour). Knopf et al. (1965) also showed an acute response in plasma GH to leucine in man. Thus, although the length of time required to elicit a response varies, the response of GH in trout is consistent with other

vertebrates.

The mechanism(s) involved in the release of GH (elevated plasma concentration) in the BCAA injected rainbow trout was not investigated. Evidence in the literature suggests the BCAA's may operate at the level of the hypothalamus affecting hypothalamic hormone release (Davis, 1972; Stewart et al., 1984). Fernstrom (1983) suggests that BCAA's influence the synthesis of brain catecholamines (CA) by competing with tyrosine, a precursor, for entry into the brain. Catecholamines directly influence GH release by either: 1) inhibiting SRIF release; 2) increasing GRF release; or 3) both (Martin, 1985), thus by inhibiting the CA may then affect SRIF and GRF release. Stewart et al., (1984) suggests if BCAA's compete with tyrosine impeding catecholamine synthesis, that the GH regulatory mechanism involved is likely: 1) influences on neural pulse-generating mechanisms; or 2) the extraneural stimulatory action of the pituitary both resulting in observed increases in plasma GH. Whether the increase in GH observed in rainbow trout is a result of such mechanisms is purely speculative.

Feeding coho yearlings with BCAA had no significant effects on growth (Fig. 2) while significantly increasing plasma GH (Fig. 6). This lack of increased growth that would be expected if GH increases is confusing and may be a result of the study animal chosen. While the primary role of GH in vertebrates may be growth, in salmonids it also participates in the physiological and metabolic adaptations associated

with smolting (Barron, 1986). Smolting describes the adaptive changes in salmonids as they prepare for migration to saltwater. During this transformation from freshwater residency as presmolts (parr) to smolts, changes in behaviour, morphology and physiology are observed (Hoar, 1976; Folmar and Dickhoff, 1980). Smolting requires changes to, and subsequent mobilization of, energy reserves in the preparatory adaptation to saltwater (Woo et al., 1978). These changes in energy reserves deplete glycogen stores (Woo et al., 1978), lipid reserves (Woo et al., 1978; Sheridan, 1984) and possibly muscle protein (Sweeting et al., 1985).

Growth hormone has a major role in the adaptation of salmonids to saltwater (Sweeting and McKeown, 1987). Sweeting et al. (1985) reports a general increase in plasma GH with the onset of smolting in coho salmon. Injections of mammalian and teleost GH in salmonids also induces morphological and physiological changes related to smolting (Donaldson et al., 1979). Sweeting et al. (1985) observed elevated glucose in coho salmon after injections of oGH and sGH and suggests glycogen stores are being mobilized. Decreases in lipid stores also occur during smolting in trout (Sheridan et al., 1985). Sheridan et al. (1985) reported that total lipid content of the dark muscle, liver, light muscle and serum are all depleted during smolting in the steelhead trout (S. gairdneri). Sheridan (1986) showed that the implantation of bGH in coho salmon parr results in

increased lipid metabolism while having no effect on smolts. This suggests GH plays a significant role in depletion of lipid reserves usually observed with the onset of smolting.

Perhaps the most notable influence of GH during smolting is its role in osmoregulation. As smolts move from the hypoosmotic freshwater environment to hyperosmotic saltwater, ionic balance becomes essential for the well being of the animal. Komourdjian et al. (1976) found pGH increased saltwater survival of Atlantic salmon (S. salar). Clarke et al. (1977) noted the saltwater adaptability of sockeye salmon (O. nerka) increased following treatments with Tilapia GH before transfer. Sweeting and McKeown (1987) report coho salmon transferred to saltwater have increased plasma GH levels of 250% within 36 hours of transfer, suggesting that the GH osmoregulatory response is rapid. The means by which GH aids osmoregulation is by increased stimulation of Na+, K+ - ATPase activity, while not influencing chloride cell density (Richman and Zaugg, 1987). The conclusion was that in osmoregulation, GH stimulates increased activity of Na+, K+ - ATPase in existing chloride cells. Bolton et al. (1987) demonstrated that GH had specific saltwater adapting actions in rainbow trout (S. gairdneri). Trout subjected to doses of sGH had reduced concentrations of plasma Na+ and Mg2+ illustrating the importance of GH during osmoregulation.

Therefore, it was concluded from the results that the increased GH concentrations observed in treated coho

yearlings was being used in metabolic and physiologic pathways as the yearlings prepared for saltwater adaptation. Although purely speculative, the literature appears to support the role of GH in smolting adding credence to this hypothesis. If this hypothesis is correct then no growth response to elevated GH would be found and perhaps even a decrease in weight may occur as GH mobilizes and depletes certain energy stores such as lipids.

Another hypothesis recently discussed by Down et al. (1988a) suggests that a minimum effective level of plasma GH is required to increase growth. Down et al. (1988b) showed that maintenance of plasma GH concentrations of approximately 100-200 ng/ml were needed in order to significantly increase growth in recombinant bGH treated coho salmon. The problem with maintaining such levels by stimulating endogenous GH release may be that: 1) receptors may be saturated early causing excess GH to be metabolized rather than increasing growth; 2) a feedback inhibitory system may shut down GH release before the minimum effective dose is reached (Down et al. 1988b).

If in fact this minimum effective concentration does exist, this may also explain the lack of growth in treated coho yearlings. BCAA stimulated a plasma GH increase of only 33.0 ng/ml which is approximately a third the minimum level identified by Down et al. (1988a,b).

Coho fry showed a positive response to supplemental feeding with BCAA increasing significantly in both weight

and length over controls (Fig. 12). This suggests the possibility of either an increased dietary requirement for the BCAA's or positive influences on the endocrine system increasing GH.

. The importance of dietary BCAA's in animal growth has been well documented (Oestemer et al., 1973; Smith and Austic, 1978; Calvert et al., 1982; Hargrove et al., 1988). Similarily, the importance of BCAA's on growth in teleosts has also been well studied (Chance et al., 1964; Mertz, 1969; Wilson et al., 1980; Robinson et al., 1984).

Chance et al., (1964) investigated the BCAA requirements of chinook salmon, and found the dietary requirement for: leucine was 1.6% of the dry diet; isoleucine 1.1% of the dry diet and was influenced by the leucine content; and valine 1.3% of the dry diet. Mertz (1969) reports similar BCAA requirements for chinook salmon with the exception of isoleucine which was reported to be 0.9%. In addition, Chance et al. (1964) showed leucine appeared to be the most important in that isoleucine requirements were influenced by leucine. Diets formulated to contain suboptimal levels of leucine and excess levels of isoleucine resulted in significantly reduced growth rates. They concluded that the total quantity of leucine and isoleucine was important to maximizing growth, as well as, the ratio of leucine to isoleucine. The greatest weight gain occurred with a ratio of leucine to isoleucine between 1.2-1.6:1 with weight gain decreasing as the ratio decreased.

The commercial diet (Ewos) used in this study contained 3.8%, 2.0% and 2.7%, leucine, isoleucine and valine respectively (Table V). These values are considerably higher than those required to optimize growth in chinook salmon (Chance et al., 1964; Mertz, 1969) and trout (Ogino, 1980) and constitute a ratio of 1.9:1, leucine:isoleucine. Supplementation of diets with crystalline BCAA increased the total available amino acids by approximately 0.3%, or 4.1%, 2.3% and 3.0% leucine, isoleucine and valine respectively.

Growth effects of excess dietary BCAA in teleosts have recently been studied using the channel catfish (Ictalurus punctatus) (Wilson et al., 1980; Robinson et al., 1984). Wilson et al. (1980) found the dietary BCAA requirements for catfish to be 0.84% leucine, 0.62% isoleucine and 0.71% valine. They also noted the level of dietary leucine directly influences the concentration of both serum isoleucine and valine. Thus, dietary leucine may have an important role in the utilization and/or the uptake of isoleucine and valine. The BCAA research in catfish is supported by investigations in other vertebrates (Clark et al., 1966; Hambraeus et al., 1976). Hambraeus et al., (1976) found diets completely lacking leucine showed increases in serum levels of both isoleucine and valine in man. The mechanism proposed by Hambraeus et al. (1976) suggested leucine was critical for the tissue uptake of the BCAA's as well as their intercellular metabolism. Harper et al. (1970) found decreased levels of leucine and alpha-ketoisocaproic

acid (keto analogue) led to reductions in the transamination and catabolism of isoleucine and valine causing plasma levels to increase. Hambraeus et al. (1976) pointed out that, although this may explain the increase in plasma isoleucine and valine, it fails to answer why dietary leucine has such a specific effect on plasma BCAA's. Wilson et al. (1980) proposed a similar role for dietary leucine in catfish. In catfish when dietary leucine levels were increased from 0.6 to 0.7%, serum free isoleucine and valine levels increased approximately 6 times (Wilson et al., 1980).

The growth depressing or limiting effects of leucine has also been well studied. Harper et al. (1954, 1955, 1970), and Hargrove et al. (1988) all showed that excess leucine inhibits growth in vertebrates and the inhibition can be alleviated by the addition of small amounts of isoleucine. Benton et al. (1956), Spolter and Harper (1961), Harper et al. (1970) and Hargrove et al. (1988) all showed a similar response to increased dietary valine, thus illustrating a possible antagonistic relationship between the BCAA's. Robinson et al. (1984) could not create this antagonistic response in channel catfish where the deleterious effect of leucine on growth was not alleviated by increasing either isoleucine, valine.

In catfish fed with supplemented basal diet containing all BCAA's both growth and feed utilization efficiency improved (Robinson et al., 1984). The increase in growth

translated into 239% when treated fish were compared to controls. They suggested from these results, that either BCAA deficiencies existed in the basal diets or that a beneficial effect of excess BCAA's may exist. In further investigations with diets containing single or paired BCAA's the addition of all BCAA's were required to increase growth.

The findings for catfish add strength to the results from the coho fry fed excess BCAA's which achieved better growth (52% increase) than the controls. The level of available BCAA's in the commercial diet was not deficient, as the BCAA's exceeded required minimum amounts set for chinook salmon (Chance et al., 1964; Mertz et al., 1969) and rainbow trout (Ogino, 1980). The growth response in coho fry appears to suggest that requirements for BCAA in coho fry may exceed published requirements for either chinook salmon or rainbow trout. In addition, the literature provides ample evidence to support the importance of all three BCAA's existing together in order to effect growth.

The existence of a positive response in coho fry and the lack of a growth response in coho yearlings adds further credence to the hypothesis of an decreased need for certain essential amino acids as the fish increase in size or age. Hargrove et al. (1988) discusses such a size dependent relationship in the cat. In this study they suggest the age and size of kittens used becomes an additional factor influencing the adverse effects of leucine on growth. Harper et al. (1970) noted that it was difficult to produce

deleterious effects of disproportionate amounts of amino acids in older rats and that studies showing adverse effects used weanling rats.

Another hypothesis for the increased growth in coho fry observed in this study may be the positive effect BCAA's have on the endocrine system and release of GH (Stewart et al., 1984). In the cannulation studies previously discussed, BCAA's injected into the circulatory system of rainbow trout significantly increased plasma GH. In coho yearlings the feeding of excess BCAA's also significantly increased plasma GH. In coho fry plasma GH could not be measured due to their size and subsequent small quantity of plasma, but the possibility of increased GH and/or sensitivity of endocrine regulatory mechanism(s) to BCAA's should not be ruled out.

As previously discussed, the ratio of leucine to isoleucine and their antagonistic interaction appears to have an important role in the influence of BCAA's on growth. In experiment 4, three different concentrations of BCAA were examined (1.3 mg/g, 3.8 mg/g, 11.4 mg/g) at two different ratios (1:1:1, 2:2:1; val:leu:ile) of the individual amino acids. The results of the various concentrations regardless of the ratio were nonsignificant differences in growth (Fig. 17 and 18).Thus, the concentration of excess dietary BCAA needed to elicit a minimum growth response may be less than the 0.3% increase (3.8 mg/g) used with coho fry in experiment 3 and requires further investigation. The comparison of growth at concentration and the two different

ratios was also found to be nonsignificant on growth (Fig. 19, 20 and 21).

4.2 Arginine Aspartate (AA)

Results reported from the injections of rainbow trout with AA revealed no significant effects on plasma GH (Table II). Treated fish were compared to controls and although the mean plasma GH in the treated fish appeared to increase at 30 minutes, the difference in GH was not significant. This lack of response is inconsistent with results found for just arginine in other vertebrates (Davis, 1972).

In sheep, Davis (1972) infused arginine at a concentration of 0.5 g/kg over 30 minutes. A peak GH response was seen 30 minutes from the start of the infusion then gradually decreasing. The concentration used by Davis (1972) was approximately twice the amount of AA injected into the rainbow trout and four times the arginine portion of AA. Therefore, the lack of a GH response may be a result of too small a treatment dose used on the rainbow trout. Another possibility for the lack of a GH response may be an artifact of the experimental design, such that an increase could have occurred before the first sample at 30 minutes.

The growth data from coho yearlings indicates that the supplemental feeding of AA has no significant effect (Fig. 3). Plasma GH data also showed no significant effects of the AA supplemented diet (Fig. 7). Franchimont et al. (1984) fed a 250 mg/kg concentration of AA and 140 mg/kg

concentration of the single amino acid arginine to rats. They found that arginine did not result in any significant GH response, whereas, AA resulted in an increase in GH, 30 minutes after administration. In a previous conflicting study (Franchimont et al., 1979) GH levels did not change following treatment with 100 mg/kg of AA, but when increased to 250 mg/kg in their more recent study (Franchimont et al., 1984) an increase was obtainable. This response in plasma GH to AA in rats ocurred at 30 minutes after administration. The AA concentration fed to coho yearlings was approximately 15 times greater than that used by Franchimont et al. (1984) and still had no apparent effects. However, blood samples were not collected immediately following feeding with AA and if short term increases in circulating GH occurred they may be missed. In addition, the dose response reported by Franchimont et al., (1984) may also prove relevant in fish and that indeed had a dose response experiment been conducted an increase GH may have been found in coho yearlings.

Correlations between the individual weight (Fig. 10) and length (Fig. 11) versus plasma GH in coho yearlings is of particular interest. This relationship shows that greater increases in plasma GH occur in smaller fish so, feeding AA may increase plasma GH at an earlier age in a manner similar to GABA which affects pituitary GH secretion in only young rats (Acs et al., 1984). Why smaller fish have higher GH concentrations in response to AA is not known. It may be a

reflection of increased pituitary secretion rates or decreased utilization by target tissues or both. In addition, because the total blood volume is less in small fish, if the blood is used as a storage site for GH, levels per millilitre could conceivably be higher.

In coho fry a positive effect on growth was achieved with significant increases in both weight and length (Fig. 13). Arginine levels in young fish has been shown to be important in achieving maximum growth (Ketola, 1982, 1983; Walton et al., 1986). It was concluded that the most important component of the AA salt was the arginine component which accounts for approximately 56% of the total molecular weight. These results, then raise the possibility that younger coho juveniles may have a higher arginine requirement than older fish in order to achieve maximum growth.

Klein and Halver (1970) determined that coho salmon required 2.4% of the dry diet be comprised of arginine in order to attain constant optimal growth. The fish used in this study were approximately 2.9 g in size and the largest percentage increase (230%) was achieved in fish fed arginine at 3.6% of the dry diet. In rainbow trout, Walton et al. (1986) found the optimum requirement for arginine was 1.6-1.8%, which was substantially lower than the 2.5-2.8% reported by Ketola (1983). It is interesting to note that although both studies were conducted on rainbow trout, the fish having the lower arginine requirement (Walton et al.,

1986) averaged 7.0 g, whereas those fish with a higher arginine requirement averaged 1.1g (Ketola, 1983). Thus, the possibility of a size relationship in arginine requirements in one species of salmonid may exist.

In the diet (Ewos) fed to coho yearlings and coho fry in this study the arginine content was found to be 2.6% of the dry diet (Table V). This is slightly higher than what was recommended by Klein and Halver (1970) as optimal for coho (2.4%), but lower than the amount found to yield the greatest percent weight gain (3.6%). The supplementation of the diet with AA (3.8 mg/g) in both experiments increased the arginine content of the diet to an average of 3.2%.

The difference in the response of yearlings and fry to AA supports the hypothesis that larger and presumably older fish may have a decreased requirement for arginine. The increased ability of coho yearlings to synthesize arginine may be one reason. Chiu et al., (1986) noted that arginine requirements found in rainbow trout varied greatly and that the existence of a full complement of urea cycle enzymes and confirmation of a urea cycle may help to explain these differences. They postulated that if the urea cycle is functional to a limited extent in larger trout, that the amount of dietary arginine required to support maximum growth may decrease. If a similar system exists in coho salmon, then they may also exhibit a decreased dietary arginine requirement as they increase in size and age.

Another hypothesis for observed increases in the coho

fry growth may be the sensitivity of newly emerged or ponded fry to certain amino acids and the effects of these on exogenous feeding. Electrophysiological and behavioural studies indicate the olfactory and gustatory senses of salmonids are sensitive to certain chemicals released from their food (Sutterlin and Sutterlin, 1970; Shparkovskiy et al., 1983; Mearns, 1985, 1986). Shparkovskiy et al, (1983) showed that the behaviour of the Atlantic salmon (S. salar) was positively influenced by aspartic acid but negatively influenced by arginine, and that these responses decreased as fish age increased. The L-amino acids appear to be stimulatory and play a role in the detection and recognition of potential food sources (Mearns, 1985, 1986). Mearns (1986) found that brown trout (Salmo trutta) showed significant positive responses to L-arginine. Therefore, by increasing the reaction of fry to introduced feed better utilization of the feed should be achieved and thus improved growth. Although the data can not support this hypothesis it should not be dismissed prematurely.

4.3 Gamma-aminobutyric Acid (GABA)

The results presented from the injections with GABA in rainbow trout show plasma GH concentrations are increased over the controls (Table III). Observed increases in plasma GH were significant at 90 minutes and maintained this increase throughout the remainder of the experiment (210 minutes). These results are both contradictary (Flok et al.,

1984) and consistent (Takahara et al., 1980; Willoughby et al., 1986) with GABA injection experiments in other vertebrates.

The systemic injections of GABA into the third ventricle of chronically cannulated rats results in inhibition of spontaneous GH release by inhibiting GRF secretion (Fiok et al., 1984). The GABA agonist muscimol and GABA metabolic inhibitors (ethanolamine-alpha-sulphate and gamma-acetylenic GABA) also resulted in similar inhibitions in GH secretion. Whereas, the GABA receptor antagonist biculline methiodide caused early secretory peaks in plasma GH (Fiok et al., 1984).

Contrary to the above inhibitory studies, Acs et al. (1984, 1987) demonstrated that GABA and its agonist muscimol stimulated GH release in vitro. Pituitary glands of neonatal rats were treated either with GABA or muscimol and the resulting GH measured. In both studies the release of GH responded in a dose dependent fashion. Willoughby et al. (1986) showed that activation of GABA receptors with muscimol, an agonist, in chronically cannulated rats increased plasma GH. The response of muscimol was dose dependent and the effective dose (0.16 nmol) elicited a 346% increase 15 minutes after injection. Also in rats, Takahara et al. (1980) administered GABA by intraventricular (i.v.) injections and aminooxyacetic acid (AOAA) (GABA catabolism inhibitor) and gamma-hydroxybutyrate (GRB) (physiological metabolite of GABA), by systemic injections causing

significant increases in serum GH.

Exactly how GABA injected into the circulatory system of rainbow trout effects the pituitary and the concommitant change in plasma GH is speculative. Presumably, the injected GABA or metabolite is transported by the systemic circulatory system across the blood/brain barrier where it acts to either: 1) release neuropeptide hormones of the hypothalamus (SRIF, GRF) or; 2) act directly on the somatotrops of the pituitary. There is also a suggestion that GABA may play a dual role in regulating the release of GH by either inhibiting the release of SRIF or GRF (Muller, 1987).

Murakami et al. (1985) found the intra-cerebraventricular injection of GABA induced an elevation in GH that could be abolished by injecting anti-rat GRF. This suggested the possible involvement of GABA in the regulation of the hypothalamic hormone GRF. Murakami et al. (1987) demonstrated that central nervous system (CNS) somatostatin induced GH secretion was inhibited by picrotoxin, a GABA anatagonist, suggesting a GABAergic mechanism in controlling SRIF. Murakami et al. (1987) concluded that (CNS) SRIF may first activate GABAergic mechanisms which may then be responsible for stimulating GRF release and subsequent increases in GH. Research in the rat appears to point to hypothalamic control of GH, with the neuropeptide hormones SRIF and GRF acting as messengers, and GABA affecting the secretion of these messengers. Nothing suggests a similar

mechanism does not exist in fish. Therefore, the increase in plasma GH observed in rainbow trout may be a result of the influence of GABA or one of its metabolites on the neuropeptide hormones of the hypothalamus.

Data from the supplemental feeding of GABA to coho salmon yearlings and coho salmon fry had conflicting results. Coho yearlings showed no effects on overall growth during the 56 day experimental period (Fig. 4), yet a significant increase in plasma GH occurred. No evidence exists to support the hypothesis that feeding crystalline GABA effects the plasma GH concentration in vertebrates, yet in coho yearlings dietary GABA does elevate plasma GH.

Although the primary role of GH is to stimulate growth, an important role in presmolts as previously discussed is the preparatory role for smolting (sect. 4.1). Therefore, if the increased GH is shunted to, or utilized by, metabolic or physiologic pathways an increase in growth may not be observed. Similarly, if a minimum effective concentration of 100 ng/ml is required (sect. 4.1) to increase growth , GABA did not achieve this (55 ng/ml).

The hypothesis of a size relationship with regards to the effectiveness of GABA on GH proved negative. The correlation and regression relationship between weight and length versus plasma GH in treated fish was non significant and not reported. In rats, Acs et al. (1984) found GABA caused GH release in vitro from the pituitaries of neonats while having no effect on adult pituitaries. The mechanism

proposed was that a protein inhibitor (GABAmodulin) in adult rats was absent from receptors on the somatotrops of neonats providing free access to binding sites and stimulation of GH. This model, supports direct pituitary stimulation by GABA either bypassing or in conjunction with, the hypothalamus. Although functional in rats, there is no evidence to support its existence in fish. If this system exists in fish, GABA may prove more effective in stimulating the release of GH and subsequent growth in younger fish (fry versus smolts).

Coho salmon fry showed a positive response to supplemental feeding with GABA significantly increasing both weight and length over controls (Fig. 14). Unfortunately, plasma GH could not be measured because of the small size of the fry. If the pattern of response to GABA is similar in fry as it was in yearlings then a significant increase in plasma GH preceeds the increase in size of the fry. This suggests a lag time of the growth response to the increased GH. Sweeting et al. (1985) found no correlation between GH and weight in presmolts until a comparison of the following week's weight (increased) was examined. Growth hormone is a strong protein anabolic hormone in mammals (Turner and Bagnara, 1976) and displays this same function in salmonids (Higgs et al., 1975). Growth in fish which involves the synthesis of muscle protein would not be expected to react immediately to GH. Rather, it would react after GH were elevated, thus explaining the lag in the growth response to

GABA observed.

4.4 Clonidine

Results reported from injections of rainbow trout with clonidine showed no significant increases in plasma GH (Table IV). These results which are nearly significant at 210 minutes (p=0.059) could be significant if the sample size were increased (n>3). This lack of response in plasma GH is inconsistent with results of clonidine injection and infusion experiments in other vertebrates (Lal et al., 1975; Chang et al., 1985; McWilliams and Meldrum, 1985; Cella et al., 1987; Muller et al., 1987).

The administration of clonidine by intravenous (i.v.) injections in man has been shown to induce the release of GH (Lal et al., 1975). Clonidine injected at 0.15 mg increased serum GH by approximately 600% in 67% of the patients treated. McWilliams and Meldrum (1985) infused clonidine at a concentration of 100 nmol/ul into the lateral or medial hypothalamus of monkeys over 5 minutes monitoring the response of GH at 5, 10 and then every 15 minutes for a total of 75 minutes. These treatments resulted in an acute response in GH with peak levels (>10 ng/ml) at 30-45 minutes, and returning to basal levels by 75 - 90 minutes after infusion. Infant rats injected i.p. with 150 ug/kg of clonidine had plasma GH levels 127% higher, 15 minutes after injection (Cella et al., 1987).

The majority of studies with clonidine in higher

vertebrates, document the response of plasma GH as acute or short term in nature, with peak GH levels occurring within 60 minutes after treatment. This type of response did not occur in rainbow trout and there is little evidence in the literature to support the hypothesis that it would. Although, Chang et al. (1985) noted the i.p. injections of clonidine (30 - 300 ug/g) in goldfish (Carassius auratus) significantly increased plasma GH. They suggest the alpha-adrenergic system does exist in goldfish and is involved with GH release but do not speculate on the mechanism.

Concentrations of clonidine injected into the rainbow trout (250 ug/kg) were 5 times the minimum amount used by Cella et al. (1987) to elicit a response in infant rats (50 ug/kg), yet 120 times less than the reported amount needed in goldfish (Chang et al., 1985). Based on the wide range of concentrations required to show a positive effect, the lack of a significant response in trout may be a result of too small a treatment dose.

Supplemental feeding with clonidine to coho yearlings did not result in any significant increases in either weight or length over controls (Fig. 5). Coho yearlings displayed an initial increase after 14 days in both weight and length but at the end of 56 days were not significantly different from controls. Plasma GH in coho yearlings treated with clonidine resulted in a rapid increase after 14 days of treatment, peaking at 28 days then decreasing (Fig. 9).

Oral clonidine treatments are a safe and reliable means

of eliciting a GH response in human children and young
adults (Gil-Ad et al., 1979). Children treated with a single
dose of clonidine (0.15 mg/m2) were found to have pronounced
plasma GH increases of 602% with the peaks occurring 60 -
120 minutes after treatment. In adults, the same dose also
produced an increase in plasma GH although not as dramatic
(81%) peaking 90 minutes after treatment (Hunt et al.,
1986). Contrary to these findings, Joffe et al. (1986) found
no GH response to clonidine in subjects first subjected to
exercise. Plasma GH response to oral clonidine treatments
appears to be acute, peaking in less than 3 hours after
administration. The effects of chronic clonidine treatment
appears not to be documented for vertebrates, while in the
coho salmon yearlings continuous chronic treatment (56 days)
with clonidine clearly elevates plasma GH.

The proposed mechanism for the response of GH to
clonidine is the activation of the adrenergic system.
Clonidine is an alpha-2-adrenergic agonist and clearly
influences GH release in vertebrates, whether administered
by injections (Lal et al., 1979; Chang et al., 1985; Cella
et al., 1987), infusions (McWilliam and Meldrum, 1985;
Bramnert and Hokfelt, 1987; Siever et al., 1987) or orally
(Gil-Ad et al., 1979; Hunt et al., 1986). The adrenergic
system encompasses the neurons and/or synapses that release
or receive the catecholamines (CA): epinephrine (E),
norepinephrine (NE) and dopamine (DA). Further separation of
the adrenergic system reveals two distinct receptor groups:

alpha and beta, which are separated on the basis of receptor properties. These receptors are broken down further into subclasses, namely; alpha-1, alpha-2, beta-1 and beta-2. Clonidine, is a pharmacological agent, acting as an alpha-2-adrenergic agonist, mimicing the catecholamines and producing a receptor response, while inhibiting the CA that would normally elicit the response.

Some evidence exists to support the role of catecholamines in GH release in one teleost species (Chang et al., 1985). In this study using goldfish, i.p. injections of L-Dopa an intermediate to NE and E increased serum GH, as did i.v.t. injections of DA. Chang et al. (1985) suggested that the results support the theory of an alpha-adrenergic mechanism involved in stimulating GH release in goldfish. Lal et al. (1979) found that clonidine administered by i.v. produces results compatible with the dual noradrenergic, dopaminergic regulatory mechanism regulating GH release in rats. Gil-Ad et al. (1979) also concluded that clonidine induces GH secretion by specific activation of alpha-adrenergic receptors. Thus, it appears clonidine is a potent alpha-2-adrenergic receptor stimulant and reacts with the postsynaptic receptors in releasing GH (Rudolph et al., 1980; Locatelli et al., 1985; Siever et al., 1987).

Further evidence of the mechanism shows that in young rats, clonidine stimulated release of GH can be suppressed by pretreatment with an antibody to GRF (Cella et al., 1987). Alternatively, treatment with clonidine and

antibodies to SRIF induces an additive response to plasma GH
levels. This led to the conclusion that alpha-2-adrenergic
stimulation caused by clonidine and resulting in GH release,
may act on the endogenous release of GRF and not SRIF. It is
the GRF from the hypothalamus that would act as a messenger
to the pituitary to release GH into the circulatory system
(Martin, 1985). In adult rats, similar results were obtained
in work by Eden et al (1981) and Kabayama et al. (1986). The
use of this mechanism of action from the rat to explain the
response observed in the salmonid is purely speculative, but
does warrant consideration until further details of the
neural endocrine control of GH in the teleost is clarified.

The hypothesis supporting the lack of an increase in
growth (Fig. 5) corresponding to the induced increase in
plasma GH has been discussed previously (sect. 4.1) and may
also have been a result of smolting in experimental fish.
Similarly, clonidine elicited only half of the minimum
effective concentration discussed previously (sect. 4.1)
which may also help to explain the lack of a growth response
in treated coho yearlings.

Coho fry treated with clonidine showed a significant
increase in both weight and length (Fig. 15) over the
controls. Unfortunately, the plasma GH levels could not be
measured due to the small size of the fry. If the assumption
is made that oral treatments with clonidine resulted in a
similar GH response as was observed in the yearlings, then
the elevation of GH corresponds to the increase in weight

and length. Sweeting et al. (1985) noted that in coho presmolts (yearlings), GH levels correlated to increases in growth the following week. This fits nicely with the argument presented above for the coho fry.

4.5 Canola Oil

The Canola oil used to coat compounds to the feed had no apparent significant effect on growth. This was substantiated in experiment 3 where the use of Canola oil had no significant effect on either weight or length of coho fry. Canola oil as an alternate or supplemental dietary lipid source for coho or chinook salmon juveniles does not affect growth rates (Dosanjh et al., 1984, 1988). Canola oil was also shown to have no influence on the ability of either coho or chinook salmon juveniles to convert food or protein into flesh, or hamper their efficiency at utilizing dietary proteins (Dosanjh et al., 1984,1988). Thus, the effects of Canola oil as a vehicle to administer, BCAA, AA, GABA or clonidine to the experimental fish in experiments 2, 3, and 4 was considered neglible.

The results of water analysis in experiment 2 revealed Canola oil was adequate for adhering the test compounds to feed pellets. The rate of compound leaching appeared to occur rapidly during the first 30 minutes then level off. Most feed consumption occurred within the first few minutes following food presentation, therefore, it was concluded that although some leaching did occur, the Canola oil held

the test compound to the feed long enough to ensure the majority was consumed by the fish. In addition, plasma analysis for the BCAA revealed that the three BCAA were elevated in those fish fed excess BCAA indicating that the compound (BCAA) was getting into the fish.

5.0 CONCLUSIONS

The purpose of this study was to attempt to enhance growth in juvenile salmonids reared under simulated aquaculture conditions. The method selected was the use of compounds known to increase GH in other vertebrates and either inject or feed these compounds to salmonids. It was postulated that if GH could be increased that a concomitant increase in growth, measurable by changes in weight and length may occur.

The results of this study led to the following conclusions:

1) BCAA and GABA injected directly into the circulatory system of rainbow trout will significantly increase plasma GH, while AA and clonidine have no effect. The mechanism involved is purely speculative but based on existing literature the increase may occur by stimulation of hypothalamic hormones or direct stimulation of somatotrops.

2) Growth measured as changes in size of coho yearlings was not affected by feeding diets supplemented with BCAA, AA, GABA or clonidine. Plasma growth hormone on the other hand significantly increased in yearlings fed with BCAA, GABA and clonidine but not AA. This stimulated increase in GH did not result in any increases in growth. It was concluded that perhaps GH was being utilized for another function such as smolting and saltwater adaptation.

3) Correlations between fish size and GH were significant only in fish fed AA. These correlations showed higher plasma

GH concentrations in smaller fish and may be a result of greater endocrine sensitivity, influencing GH release.

4) Coho fry significantly increased in both weight and length after being fed BCAA, AA, GABA and clonidine. These results with respect to BCAA and AA may suggest a higher requirement for specific essential amino acids in younger fish.

5) An attempt to find a more suitable concentration and ratio for supplemental BCAA was unsuccessful. Results suggest that an amount less than 1.3 mg/g BCAA at a ratio of 2:2:1 may be all that is needed to solicit an increase in fry growth.

Generally, it was concluded that it is possible to increase plasma GH and growth in juvenile salmonids using a group of select compounds. These compounds which may or may not be feasible in a production setting warrant further investigation.

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