SEX STEROID-INDUCED INHIBITION OF FOOD INTAKE IN SEA BASS (*Dicentrarchus labrax*).

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Running Title: Sex steroids and food intake in fish

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ABSTRACT

This study was conducted to test the sensitivity to gonadal steroids of the systems regulating food intake in sea bass. Animals were treated with silastic implants containing 17-β-estradiol or testosterone. Self-feeding was recorded for 31 days using computerized demand feeders and unfed-pellet recovery systems. Both steroids strongly decreased self-feeding levels, feed efficiency and specific growth rates. The linear growth of fish treated with testosterone was higher than in 17-β-estradiol treated fish. In the second experiment, fish were treated with lower 17-β-estradiol doses and 11-keto-androstenedione, a precursor of the main fish androgen (11-keto-testosterone). The results demonstrated a dose-response effect of estrogen and no effect of non-aromatizable androgens on food intake or growth performance. The inhibitory effect of testosterone on food intake seems to be mediated by its aromatization to estradiol, while linear growth promotion is mediated by the androgen "per se". Data suggest that gonadal steroids may be involved in the seasonal feeding pattern of sea bass. The results demonstrate the sensitivity of the mechanisms regulating food intake to estrogenic compounds and point to the risk of including feed containing estrogenic substances in fish diets as well as the risk involved in exposure to "estrogenic environments".

Keywords: Estradiol-Estrogen, Testosterone-Androgen, Phytoestrogen, Feeding Behaviour, Fish Nutrition.

INTRODUCTION

The control of food intake involves multifaceted interplay between diverse hypothalamic neuronal systems together with their interaction with reporter systems that convey peripheral information to the central neuronal systems (reviewed by Volkoff et al., 2005). Several studies have demonstrated that the peripheral endocrine information provided by reproductive tissue is involved in the physiological control of appetite in mammals (reviewed by Eckel, 2004). In fish, data regarding the involvement of the sex steroids in the control of food intake and growth are controversial. Early studies in salmonid (reviewed by McBride et al., 1982) and cyprinid (Lone 1989 and references therein) species demonstrated that both gonadal and synthetic androgens are effective anabolic agents when administered in the diet. Results obtained in the red sea bream (*Chrysophrys major*) revealed that testosterone exerts its anabolic action by increasing appetite, food conversion efficiency, specific growth rate and the activities of digestive enzymes (Woo et al., 1993). Subsequent experiments in tilapia (*Oreochromis niloticus*) further suggested that the endogenous androgens, testosterone and 11-keto-testosterone (11-K), are responsible for the higher growth rate in males than in females (Toguyeni et al., 1997). The response to external androgens seems to be species-dependent since experiments in the channel catfish (*Ictalurus punctatus*) showed that the oral administration of 17-α-methyltestosterone deterred the growth of juvenile animals (Simone, 1990). Similar results using cocoa implants of 17-α-methyltestosterone have recently been reported in the Eurasian perch (*Perca fluviatilis*), in which reductions in the food intake level and food efficiency were responsible for the decreased growth rate (Mandiki et al., 2004). In contrast, the dietary

administration of testosterone did not affect the growth rate in the above species but significantly attenuated the food intake with the concomitant increase in the food utilization (Mandiki et al., 2005). Similarly, dietary 17-α-methyltestosterone did not affect the growth of fingerling sunshine bass (*Morone chrysops* X *Morone saxatilis*, Davis and Ludwig, 2004).

The effect of synthetic and ovarian estrogens on the control of food intake and growth in fish is also controversial. Early studies in marine flatfish (*Pleuronectes platessa*) using diethylstilboestrol, a synthetic compound with oestrogenic properties, demonstrated that the lowest tested doses of the estrogen induced a marked increase in growth by stimulating food intake and the improved utilization of food protein. Higher doses caused a slight depression of growth (Cowey et al., 1973). On the contrary, low doses of diethylstilboestrol induced adverse effects on the growth rate in the channel catfish (Bulkley, 1972) and rainbow trout (*Oncorhynchus mykiss*, Matty and Cheema, 1978). Subsequent studies in the eel (*Anguilla anguilla*) demonstrated that fish treated with dietary17-β estradiol exhibited higher weight gain than the control fish (Degani, 1986; Tzchori et al., 2004). However, dietary 17-β estradiol decreased growth in the sunshine bass (Davis and Ludwig, 2004) and Atlantic halibut (*Hippoglossus hippoglossus*, Hendry et al., 2003) but did not affect appetite, food conversion efficiency or the specific growth rate in the red sea bream (Woo et al., 1993). Similar results using cocoa implants were obtained in the Eurasian perch (Mandiki et al., 2004), in which the highest dietary dose had negative effects on specific growth rate and food efficiency but no effect on food intake. These results were not reproducible since subsequent experiments using similar doses of 17-β estradiol showed an increased food intake level and enhanced specific growth rate in female Eurasian perch but

not in males (Mandiki et al., 2005).

The sea bass (*Dicentrarchus labrax*) is a carnivorous species of special interest for Mediterranean aquaculture. Formulated diets for carnivore species contain high levels of protein and oil derived from fish meal and fish oil from capture fisheries. In many species the input of wild fish biomass exceeds the farmed fish produced. In this respect, the culture of carnivorous species is considered as a net fish consumer practice rather than net producer (Naylor et al., 2000). The replacement of fish meal and fish oils in aquafeeds by plant equivalents is a priority for the long-term sustainability of these industries (Drew et al., 2007; Gatlin et al., 2007; Glencross et al., 2007). Soybean meal is the main source of vegetable protein present in animal diets, although a large number of studies have shown that a high dietary percentage of soybean meal results in decreased growth and feed efficiency in fish (reviewed by Drew et al., 2007). The poor growth rate exhibited by trout fed diets rich in soy flour has been attributed to the presence of estrogenic isoflavones, e.g. daidzein and genistein, in the bile of these fish (Kausik et al., 1995). Non-steroidal estrogenic substances are widely distributed among potential plant derived feeds (Francis et al., 2001) and fish diets (Pelissero and Sumpter 1992; Matsumoto et al., 2004). These estrogenic substances have been seen to induce behavioral changes (Clofelter and Rodríguez 2006) and alterations in the reproductive physiology of fish fed with commercial diets (Pelissero and Sumpter 1992). It is therefore possible that these estrogenic substances present in the plant-derived feeds have adverse effects on fish food intake and growth. This study represents ongoing efforts directed at understanding the contribution of estrogenic compounds in the food intake regulation of the sea bass. In a first approach, we study the effect of gonadal steroids on sea bass food intake and growth performance using self feeding systems coupled to unfed-pellet recovery systems.

MATERIAL AND METHODS

Animals

Two-year old immature sea bass [body weight (BW) = 271.66±5.01 g and length (L) = 28.45±0.15 cm] were maintained in 2000-litre tanks supplied with continuously aerated running sea water and equipped with automatic feeder activated by a string sensor placed 3 cm below the water surface. The feeders were connected to a computer system that recorded the date, the time and the tank from which each food demand originated. The feed reward per sensor activation was set at approximately 1 g/demand. Uneaten pellets were collected using a funnel (35 cm diameter) placed 30 cm down the string sensor. The funnel was connected to a flexible pipe and the uneaten pellets were discarded daily during the acclimation period. Animals were maintained under natural conditions for one year. Fish self-fed with a commercial diet (Mistral 21, Proaqua Nutrición, S.A.; 43% protein, 23% fat, 20% carbohydrates, 6% ash, gross energy 22.5 kJ/g, 3 mm standard pellet). Before the experiments, fish were placed in the experimental 500-l tanks, continuously supplied with running seawater and provided with identical self-feeding and unfed pellet collection systems, and acclimated for one month. The experimental tanks were visually isolated from the remaining tanks in the culture facilities so that routine activities did not disturb the fish. No access to the experimental area was allowed, except for the daily collection of uneaten pellets and samplings. Animals were anesthetized in 2-phenoxy-ethanol (0.1%) for 2 min before any manipulation. At the end of the experiment animals were sacrificed by rapid decapitation.

Implant procedure

Steroid implants were prepared according to Pankhurst et al., (1986) using liquid silicone rubber kindly supplied by Down Corning. Briefly, the steroid was mixed with the liquid silicone, spread in a steel mould and cured at 150 C for 90 minutes. After manufacture, implants were quickly washed in ethanol 100 % (Panreac), dried on blotting paper and stored at RT until used. 17β-estradiol (Sigma) implants were prepared at two different concentrations, 10mg/cm and 2mg/cm, whereas testosterone (Sigma) and 11-keto-androstenedione (Steraloids) implants were prepared at 10mg/cm.

Before implantation, animals were anaesthetized and a 1-1.5 cm length of blank or steroid silicone strip was implanted thorough an intraperitoneal incision of approximately 1 cm. Fish were subsequently treated with povidone-iodine gel and returned to their home tank.

Experimental Design and Procedure

Two experiments were designed to evaluate the effects of steroid implantation on sea bass food intake and growth. Both experiments were performed during the pre-gametogenic period that begins in June and ends in September. During this period, the gonads are still immature and sex steroid levels are minimal (Prat et al., 1990). Male and female fish were used indistinctly. The first experiment (Experiment 1, from May 16 to June 27, 2007), was designed to evaluate the effect of gonadal steroids (17β-estradiol and testosterone) on sea bass food intake and growth parameters. One hundred sea bass (BW = 252.4±2.54 g and L = 27.27±0.09 cm) were distributed in ten 500-l tanks (10 fish / tank). Temperature ranged naturally between 18.1 and 22.9 ºC. Self-feeding data were recorded on nine consecutive days in order to establish a base line. Subsequently (June 25, 2007), animals from four

tanks were implanted with blank implants (control treatment, n=4 tanks), 15 mg of testosterone (T treatment, n=3 tanks) or 15 mg of 17-β estradiol (E treatment, n=3 tanks) to give approximate steroid doses of 60 μg/g BW. To monitor the steroid plasma levels, blood samples from 5 animals/treatment were extracted by caudal puncture one week after implantation. Plasma was stored at -20 ºC until assayed. At the end of the experiment, animals were sampled to record biometrical parameters and blood samples were taken from 10 animals/treatment as above. Food demands were registered during thirty three consecutive days. Uneaten pellets were collected daily at 13.00h and desiccated at 100 ºC overnight. At the end of the experiment, the total amount of food distributed was calculated by weighing the food remaining in the food hoppers. This quantity was used to calculate the delivery rate for each electronic feeder. The daily delivered food was calculated using the feeder delivery rate and the number of daily demands. Finally, the daily food intake was calculated as the difference between delivered and uneaten food (mg dry weight).

The second experiment (Experiment 2, from July 11 to August 23) was designed to study the effect of non-aromatizable androgens on food intake as well as evaluate the dose-response effect of the 17β-estradiol implantation. One hundred fish (BW= 235.59 ± 2.92 g and L= 26.97±0.11 cm) were distributed in ten 500-l tanks (10 fish / tank). Self-feeding data were recorded for eight consecutive days (base line). Subsequently (July 19, 2007), animals from three tanks were implanted with blank implants (control treatment, n=3 tanks), 2 mg of 17β-estradiol (E10 treatment, n=3), 12 mg of 17β-estradiol (E50 treatment, n=2) and 12 mg of 11K-androstenedione (A50 groups, n=2). Expected steroid doses were 10 μg/g BW and 50 μg/g BW for 17β-estradiol, respectively, and 50 μg/g BW for 11K-androstenedione. Food demands were registered for thirty five consecutive days. The

sampling procedure was the same as above. Temperature ranged naturally between 22.8-25.5 ºC

Hormone analysis

Testosterone, estradiol (Neogen Corporation) and 11KT (Cayman Chemicals) plasma levels were measured by ELISA according to the manufacturer's instructions.

Expression of results and statistical analysis

Data concerning food intake, biometrical parameters and plasma hormone levels are expressed as means ± standard error. Specific growth rates were calculated as $g(X) = 100^* [(\ln X_F - \ln X_0)/t]$. X_F and X_0 indicate the value of the variable [body weight (BW), length (L) or condition factor (CF)] at the end ($_F$) and beginning ($_0$) of the experiment, respectively. Condition factor was calculated as BW (g) / L^3 (cm). Feed efficiency (FE) was calculated as total food intake / (B_F-B_0). B indicates biomass. Differences were analyzed by one-way analysis of the variance (ANOVA) followed by Tukey´s multiple range test ($P<0.05$).

RESULTS

Effective hormonal doses and plasma levels

After implantation sampling, the effective hormone doses were calculated according to the fish weight. In experiment 1, doses for 17-β estradiol (E treatment) and testosterone (T

treatment) were 58.21±1.02 and 60.49±1.08 μg/g BW, respectively. In experiment 2, the effective doses for 17-β estradiol were 41.37±1.31 and 8.67±0.18 μg/g BW for the E50 and E10 treatments, respectively. Dose for 11K-androstenedione (A50 treatment) was 45.04±0.79 μg/gBW (Table 1).

The implantation of 17-β estradiol always induced a significant increase in hormone plasma levels during the first week. After 5 weeks, plasma levels were slightly higher in experiment 2 but not in experiment 1 than in the control treatment (Figs 1A, 2A). Testosterone implantation caused a significant increase in estradiol plasma levels after 1 and 5 weeks (Experiment 1, Fig. 1A). Using similar doses to those used in the estrogen implants, the testosterone implants induced an increase in plasma hormonal levels 5 times higher than that observed in animals implanted with 17-β estradiol (Fig. 1A,B) 1 and 5 week post-implantation. Plasma testosterone levels had decreased 5 weeks post-implantation, but were significantly higher than the control levels. 11 KT plasma levels in 11K-androstenedione implanted animals were significantly higher than in control animals both 1 and 5 weeks post-implantation (Fig. 2B). However, testosterone levels in 11K-androstenedione implanted animals were similar to those exhibited in the control animals, although a slight but significant increase in the testosterone plasma levels was observed 1 week post-implantation (Fig. 2C). Estrogen levels in 11K-androstenedione implanted animals were similar to those observed in the control fish (Fig. 2A).

Effect of steroid on sea bass food intake and growth parameters

No significant differences in food intake (Figs 3A, 4A) or specific growth rate (data not shown) between groups were detected prior to the treatments. Similarly, no differences in

the daily food intake levels were detected in the control group during the whole experimental period (data not shown).

The administration of the higher doses of estrogens or aromatizable androgens induced a significant decrease in the cumulative and daily food intake levels (Figs 3A,B; 4A,B) as well as in feed efficiency (Table 1). Similarly, fish implanted with the lower dose of 17-β estradiol and 11K-androstenedione exhibited a decrease in the cumulative and daily food intake but inhibition levels did not reach statistical significance (Fig 4A,B). Feed efficiency did not show significant differences in fish treated with the above steroids compared to control fish.

Control fish were significantly heavier and longer than those treated with the higher doses of 17-β estradiol or testosterone. Accordingly, fish treated with the latter steroids exhibited lower specific growth rates in weight (g_BW) and length (g_L) than those exhibited by the control fish. The percentage of daily change in the condition factor (FC) was lower in fish treated with testosterone than in the control or 17-β estradiol treated fish, although any difference was not statistically significant. Fish treated with the lower dose of 17-β estradiol showed similar to the control fish but the 11K-androstenedione treatment induced a decrease in body weight, but not in length, compared with the control fish (Table 1).

DISCUSSION

The results demonstrate that the implantation of 17-β estradiol induces a dose-dependent decrease in the self-feeding levels, feed efficiency and growth rates in sea bass. Specific growth rates in body weight (g_BW) and length (g_L) in animals treated with the highest

17-β estradiol doses (58.21±1.02 μg/g BW) were 38.7% and 25.9%, respectively, than those observed in control animals (experiment 1). Differences in food intake were statistically significant at 15 days post-implantation. Results obtained in experiment 2 using lower doses (41.37±1.31 μg/g BW) corroborated these results, showing that estrogen-treated fish grew 47.5 (g_BW) and 49% (g_L) less than the control fish. In the latter experiment, significant differences in the food intake levels were obtained at 22 days post-implantation. Fish implanted with the lowest 17-β estradiol doses (8.67±0.18 μg/g BW) daily ate 10 % less than the control group but differences did not reach statistical significance. This difference, also observed in the cumulative food intake levels, did not cause differences in growth compared with the control group. Dose-response and time-course results suggest that this tendency in the self-feeding behaviour may result in a retarded growth rate when exposure to low doses of estrogen is prolonged. However, more experiments are required to confirm this hypothesis.

Experiments in several teleost species have demonstrated that dietary or implanted estrogens decrease growth rates by inhibiting food intake (see Introduction for references). However, this effect seems to be species-dependent since oral administration has a positive effect on growth in several eel species (see Tzchori et al., 2004 for references). The severe decrease in the demand feeding levels seems to be responsible for the observed growth retardation in the sea bass treated with the highest doses of 17-β estradiol. The estrogen implant induced a rapid increase in blood hormone levels that was resumed after 5 weeks. However, the food intake levels of implanted animals remained lower than those observed in the control fish when plasma hormone levels were similar. This persistent inhibition may indicate that the inhibitory effects are mediated by damage or temporary/permanent

reorganization of the neuronal mechanisms controlling food intake. Experiments in rat have reported neurotoxicity of the 2-methoxyestradiol, a product of 17β-estradiol metabolism in the brain (Picazo et al., 2003). Estrogens have also been reported to induce changes in the synaptology of the arcuate nucleus, a key area for the energy balance regulation in mammalian species (Hung et al., 2003). Hyperestrogenemia is used as a reversible anorexia model, providing a sustained decrease in food intake and body weight without overt toxicity in male rats (Mystkowski et al., 2000). Our experiments cannot support the reversibility of the estrogen effect on the sea bass food intake and more experiments expanding the post-implantation period or modifying the estrogen administration protocol should be designed to corroborate the irreversibility/reversibility of the estrogen-induced anorexia. Although our experimental design cannot discriminate whether the observed effects are specifically mediated by estrogen receptors, the time elapsed between the treatment and the response suggests that classical estrogen receptors working through genomic actions are involved in the observed response. Feeding behaviour and, by extension, food intake levels are regulated in the central nervous system, where the neuronal hypothalamic circuits integrate visceral and sensorial incoming information to orchestrate an integrated feeding response. It is generally accepted that the inferior hypothalamic lobe, particularly areas close to the lateral recess, as well as the ventro-posterior hypothalamus, are involved in the control of feeding behaviour (reviewed by Volkoff et al., 2005). Cloning experiments have demonstrated that the sea bass express three estrogen receptor (ER) subtypes, i.e. ERα, ERβ and ERγ (Halm et al., 2006). Our recent *in situ* hybridization studies have demonstrated that ERα is abundantly expressed within the preoptic area, tuberal hypothalamus and inferior hypothalamic lobe, suggesting

its involvement in the control of food intake in the sea bass (Muriach et al., 2008). The use of selective estrogen receptor agonist in ovariectomized rats suggests that ERα mediates the attenuating effects of estrogen on food intake and body weight gain, while ERβ has no evident effect on these variables (Roesch, 2006).

The testosterone implantation, using similar doses to those used in the 17β-estradiol implantation experiments, resulted in a 5-fold increase of plasma steroid levels. The observed plasma differences may be explained by a differential release rate related to the differences in the molecular structure or by a distinct plasma clearance rate. After 5 weeks, testosterone levels (7.3 ±0.9 ng/ml) were 30 times higher than those observed in the control animals (0.22 ± 0.034). However, after the same period, the plasma levels of 17β-estradiol were similar to those exhibited by the control animals, which suggests that the hormonal implants contain a sufficient quantity of hormone to induce plasma differences 5 weeks post-implantation. However, after this time period, the plasma estradiol clearance rate matches the hormonal release rate, resulting in no plasma differences. Studies in rainbow trout reported that the metabolic clearance rate of 17-β estradiol changes according to the reproductive status (Baroiller et al., 1987). A high metabolic clearance rate was suggested as being responsible for the low levels of plasma estradiol observed in steroid-treated female brook trout (*Salvelinus fontinalis*, Schafhauser-Smith and Benfey, 2003). The observed inhibitory effects of the testosterone implantation on the sea bass food intake and feed efficiency were similar to those reported for the 17β-estradiol treatment. Whereas specific growth rate as body weight (g_BW) was similar to that recorded in the 17β-estradiol treated fish, the specific growth rate in length (g_L) was significant higher, although below that observed for the control fish. Promotion of growth rate in length is also

evidenced by the lower change rate in the condition factor than that exhibited by the control and 17β-estradiol treated fish. The data reveal that the testosterone treatment induced a severe reduction in food intake, similar to that induced by the 17β-estradiol treatment, but stimulated linear growth compared to 17β-estradiol treated fish. Early experiments in rainbow trout demonstrated that anabolic androgens, i.e. dimethazine and norethandrolone, promote fish growth by increasing amino acid incorporation into skeletal muscle protein (Matty and Chema, 1978). Revealingly, testosterone implantation induced an increase of the 17β-estradiol plasma levels after 1 and 5 weeks. We hypothesize that testosterone aromatization to estradiol is responsible for the observed decrease in the food intake levels and food efficiency while testosterone "*per se*" promotes the stimulation of length growth. However, our *in vitro* experiments have shown that high doses of testosterone can induce ERα-mediated transcription in sea bass (Muriach et al, 2008). Therefore, it cannot be ruled out that the high effective doses of plasma hormone in the testosterone treated animals may activate the estradiol receptor to inhibit food intake and decrease feed efficiency.

To discriminate testosterone effects on food intake and feed efficiency, sea bass were treated with a non-aromatizable androgen (11K-androstenedione). The 11K-androstenedione is an immediate precursor in the biosynthesis of 11-keto testosterone (11KT), the major androgen in the fish testes (Borg, 1994) through its conversion by the enzyme 17β-hydroxysteroid dehydrogenase (17β-HSD), which is present in the testis and other tissues in fish (Mayer et al., 1990; Schulz and Blüm, 1991). Similar to testosterone treatment, 11K-androstenedione implantation strongly increased 11KT plasma levels 1 and 5 weeks post-implantation. Hence, 11KT levels (7.87 ±0.816 ng/ml) were 14 times higher than those observed in the control animals (0.56 ± 0.171) after 5 weeks. A similar increase

in plasma 11KT levels after 11K-androstenedione implantation has been reported in several teleost species (Mayer et al., 1990; Schulz and Blüm, 1991; Schmitz and Meyer, 1993; Agulleiro et al., 2007). Our results demonstrate that the food intake levels and feed efficiency in 11K-androstenedione treated animals were similar to those observed in the control fish. High doses of 11KT do not activate the ERα receptor (Muriach et al., 2008). Therefore, our data further suggest that the effect of testosterone on the sea bass food intake and feed efficiency is mediated through its aromatization to estradiol. Supporting this hypothesis, the presence of aromatase activity (González and Piferrer, 2003) and expression (Blázquez and Piferrer, 2004) in the brain and peripheral tissues of the sea bass has been reported. Treatment with non-aromatizable androgens attenuated the weight gain compared to the control group although the differences in the specific growth rate (g_BW) did not reach statistical significance. Feed efficiency was not reduced in these animals compared to the control fish. Revealingly, 11K-androstenedione did not stimulate length growth as testosterone did. We cannot explain this discrepancy, although differences in the experimental androgens could account for the observed results.

The physiological relevance of these results is uncertain. Studies in rat have demonstrated that estradiol exerts a potent physiological inhibitory effect on feeding. Food intake reductions are expressed as reductions in meal size not in meal frequency. The effect of estradiol is prominent in females during the estrous phase of the ovarian cycle compared with diestrous and proestrous phases. Meal size and body weight are increased in ovariectomized rats but regular in ovariectomized rats treated with physiological concentrations of estradiol (reviewed by Eckel, 2004). The sea bass, a seasonal spawner reaches sexual maturity at two and three years of age in both male and females. During

autumn-early winter (October-December) gonadal development occurs coinciding with the increases in plasma sex steroids and luteinizing hormone, with maximum levels reached at spawning period. The maximum food intake period of this species appears to be in spring to early autumn (May-October). Food intake gradually declines during the period of gonadal development to reach minimal values at spawning time. Decreased temperatures decline in winter are mainly responsible for the food intake attenuation during the spawning season (Sánchez-Vázquez et al., 1998; Cerdá-Reverter et al., 1999). However, our results suggest that the progressive increase in plasma sex steroids during the reproductive cycle may be involved in the anorexia observed during the late reproductive period. Accordingly, it has been reported that sex-hormone binding globulin plasma levels markedly decrease during winter months, thus increasing plasma steroid availability for tissues, including the central nervous system. Such a winter decrease seems to be related to nutritional or metabolic effects in relation to water temperature or food intake, rather than changes in gonadal sex steroid production (Miguel-Queralt et al., 2007). Androgen plasma levels after implantation during the first week were always higher than those observed throughout the reproductive cycle in the male and female sea bass but fall to the physiological range after 5 weeks (Prat et al., 1990). On the contrary, estrogen levels in both testosterone and 17-β estradiol implanted fish were similar or slightly higher than those reported in females during the reproductive period. These high androgen levels did not seem to affect fish condition and no mortality was observed in any experimental treatment. However, more studies involving the analysis of the annual feeding cycle in gonadectomized sea bass males and females are needed to corroborate the participation of the sex steroids in the anorexia observed during the late reproductive period.

Experiments in fish have demonstrated that exposure to estrogenic compounds, including phytoestrogens, may induce behavioural, morphological and physiological changes (Sumpter, 2005). Non-steroideal estrogenic substance are widely distributed among plant-derived feeds and considered as antinutritional factors in fish diets (Francis et al., 2001). The replacement of fish meal by plant-based protein sources has dramatically increased the incorporation of non-steroideal estrogenic substance, mainly phytoestrogens in fish diets (Pellisero and Sumpter, 1992; Miyahara et al., 2003). Genistein, a soybean isoflavone, decreases food intake and body weight (Kim et al., 2006; Cave et al., 2007) and promotes changes in several metabolic hormones (Szkudelska and Nogowski, 2007) in mammals. The physiological effects of phytoestrogens are not exclusively mediated by binding to the estrogen receptors (Miyahara et al., 2003) since the main soybean isoflavones inhibit estradiol metabolism (Ng et al., 2006) and the aromatase complex activity in salmonid fish (Pelissero et al., 1996). There are numerous reports of phytoestrogens, particularly genistein, exerting estrogenic effects in fish, including striped bass (Pollack et al., 2004), but studies concerning the food intake regulation in fish are scarce. Genistein inhibited weight gain in yellow perch (*Perca flavescens*, Ko et al., 1999) as did 17-β estradiol, but no effects were reported in eel (Tzchori et al., 2004). Our results suggest that the food intake levels and growth rates of the sea bass may be sensitive to the fish meal substitution by plant-derived prime materials containing estrogenic compounds.

In conclusion, we demonstrate that 17-β estradiol implantation induces a dose-dependent attenuation of food intake and feed efficiency, and inhibits sea bass growth. Testosterone administration inhibits food intake in a similar way to that recorded for 17-β estradiol implanted fish. Testosterone effects on food intake are probably mediated by central

aromatization to estradiol since the administration of non-aromatizable androgens had no effect on the sea bass food intake. However, linear growth in fish treated with testosterone increased when compared to 17-β estradiol implanted fish, suggesting that testosterone exerts anabolic effects on muscle growth independently of energy availability. Future studies will focus the physiological relevance of steroid-induced food intake inhibition as well as the effects of steroidogenic compounds on the sea bass energy balance.

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FIGURE LEGENDS

Figure 1

Time-course effects of intraperitoneal implantation of 17-β estradiol and testosterone on plasma hormonal levels in the sea bass (Experiment 1). A) 17-β estradiol plasma levels. B) testosterone plasma levels. Blood samples were taken 1 and 5 weeks post-implantation. Each point represents the mean of at least 5 determinations of individual plasma samples (±SEM). Asterisk indicates significant differences after ANOVA followed by Tukey´s multiple range test, $P<0.05$).

Figure 2

Time-course effects of intraperitoneal implantation of 17-β estradiol and 11-keto-androstenedione on plasma hormonal levels in the sea bass (Experiment 2). A) 17-β estradiol plasma levels. B) testosterone plasma levels. Blood samples were taken 1 and 5 weeks post-implantation. Each point represents the mean of at least 5 determinations of individual plasma samples (±SEM). Asterisk indicates significant differences between control and treated fish after ANOVA followed by Tukey´s multiple range test ($P<0.05$).

Figure 3

(A) Cumulative food intake level after 17-β estradiol and testosterone intraperitoneal implantation in sea bass. Differences were detected at 15 days post-implantation. Each point represents the mean ± SEM of food intake levels of at least 3 tanks containing ten animals. B) Daily mean food intake after 17-β estradiol and testosterone intraperitoneal

steroid implantation in sea bass. Each bar represents the mean + SEM of 93 (T50 and E50) or 124 (CTRL) determinations. Asterisk indicates significant differences between control and treated fish after ANOVA followed by Tukey´s multiple range test ($P<0.05$).

Figure 4

(A) Cumulative food intake level after 17-β estradiol and 11-keto-androstenedione intraperitoneal implantation in sea bass. Differences were detected 22 days post-implantation. Each point represents the mean ± SEM of food intake levels of two (A50 and E50) or three (CTRL and E10) tanks containing ten animals. B) Daily mean food intake after 17-β estradiol and 11-keto-androstenedione intraperitoneal steroid implantation in sea bass. Each bar represents the mean + SEM of 70 (A50 and E50) or 105 (CTRL and E10) determinations. Asterisk indicates significant differences between control and treated fish after ANOVA followed by Tukey´s multiple range test ($P<0.05$).

TABLE 1. EFFECT OF STEROID IMPLANTATION ON SEA BASS GROWTH

EXPERIMENT 1

Treatment	Dose (μg/gBW)	BW (g)	L (cm)	CF	g_BW (%)	g_L (%)	g_CF (%)	FE
CTRL	0	331.9±5.17**a**	29.01±0.133**a**	1.35±0.012	0.84±0.02**a**	0.19±0.002**a**	0.27±0.021**a**	1.38±0.06**a**
T50	60.49±1.08	279.5±5.26**b**	28.05±0.152**b**	1.27±0.019	0.32±0.04**b**	0.09±0.006**b**	0.02±0.059**b**	2.13±0.27**b**
E50	58.21±1.02	289.2±6.32**b**	28.01±0.178**b**	1.31±0.015	0.33±0.05**b**	0.05±0.015**c**	0.16±0.018**a**	2.35±0.05**b**

EXPERIMENT 2

Treatment	Dose (μg/gBW)	BW (g)	L (cm)	CF	g_BW (%)	g_L (%)	g_CF (%)	FE
CTRL	0	342.3±6.25**a**	29.71±0.38**a**	1.32±0.137	1.01±0.04**a**	0.26±0.194**a**	0.29±0.065	1.65±0.04**a**
E50	41.37±1.31	301.6±9.64**c**	28.35±0.26**b**	1.33±0.152	0.62±0.12**b**	0.13±0.071**b**	0.23±0.090	2.32±0.02**b**
E10	8.67±0.17	334.5±7.79**ab**	29.32±0.21**ab**	1.33±0.033	0.99±0.038**a**	0.23±0.164**a**	0.31±0.033	1.54±0.09**a**
A50	45.04±0.79	307.5±6.08**bc**	28.82±0.19**ab**	1.28±0.0283	0.97±0.055**a**	0.24±0.113**a**	0.19±0.060	1.69±0.04**a**

Data are expressed as means ± standard error. Specific growth rates were calculated as $g(X) = 100^* [(\ln X_F - \ln X_0)/t]$. X_F and X_0 indicate the value of the variable [body weight (BW), length (L) or condition factor (CF)] ant the end ($_F$) and beginning ($_0$) of the experiment, respectively. Feed efficiency (FE) was calculated as total food intake / (B_F-B_0). B indicates biomass. Different letters in the same column indicate significant differences after ANOVA followed by Tukey´s multiple range test ($P<0.05$).

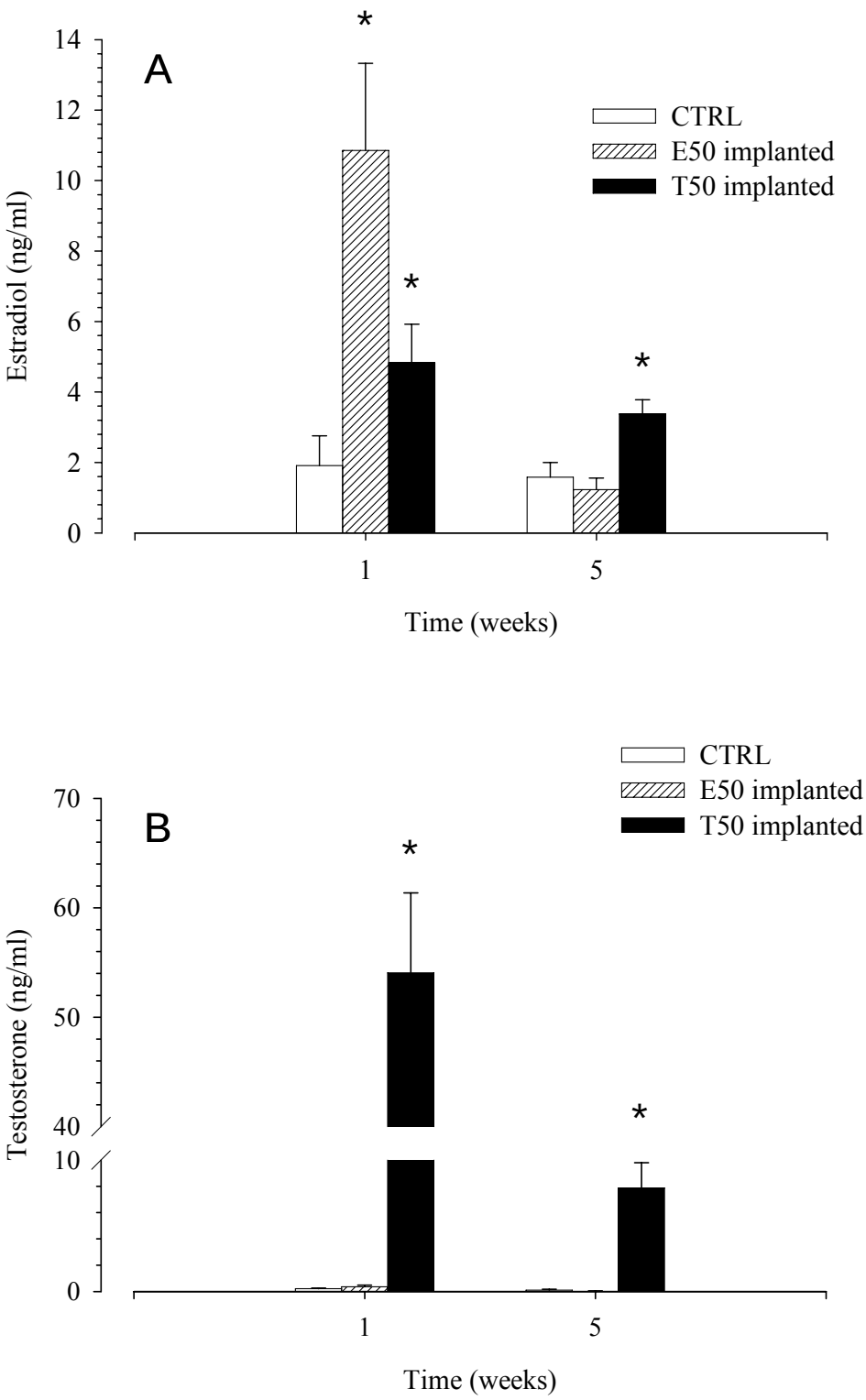

A
B
CTRL
E50 implanted
T50 implanted
Estradiol (ng/ml)
Testosterone (ng/ml)
Time (weeks)
1
5
0
2
4
6
8
10
12
14
40
50
60
70
*

Figure 1

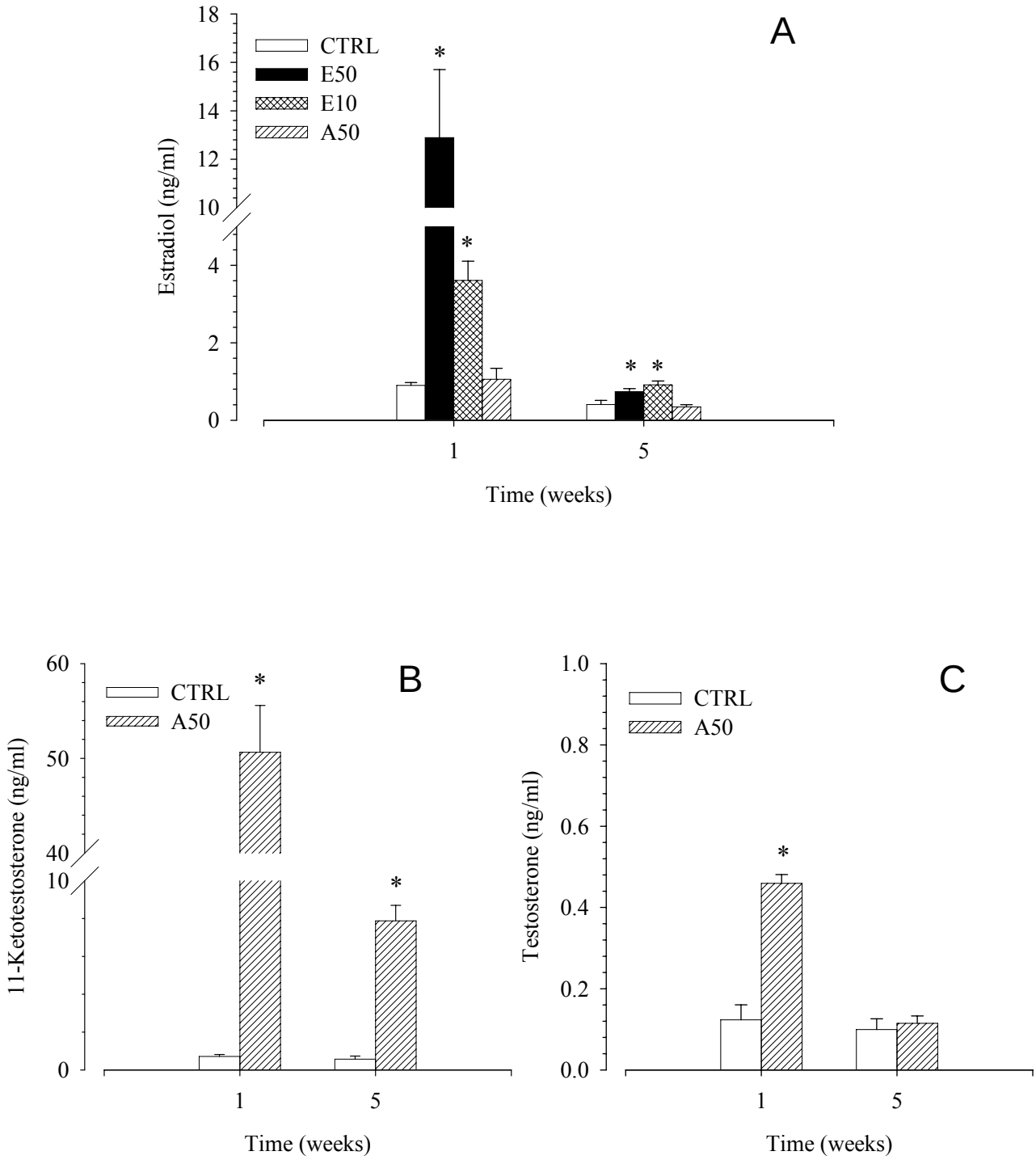

A
CTRL
E50
E10
A50
Estradiol (ng/ml)
18
16
14
12
10
4
2
0
1
5
Time (weeks)
B
CTRL
A50
11-Ketotestosterone (ng/ml)
60
50
40
10
0
1
5
Time (weeks)
C
CTRL
A50
Testosterone (ng/ml)
1.0
0.8
0.6
0.4
0.2
0.0
1
5
Time (weeks)

Figure 2

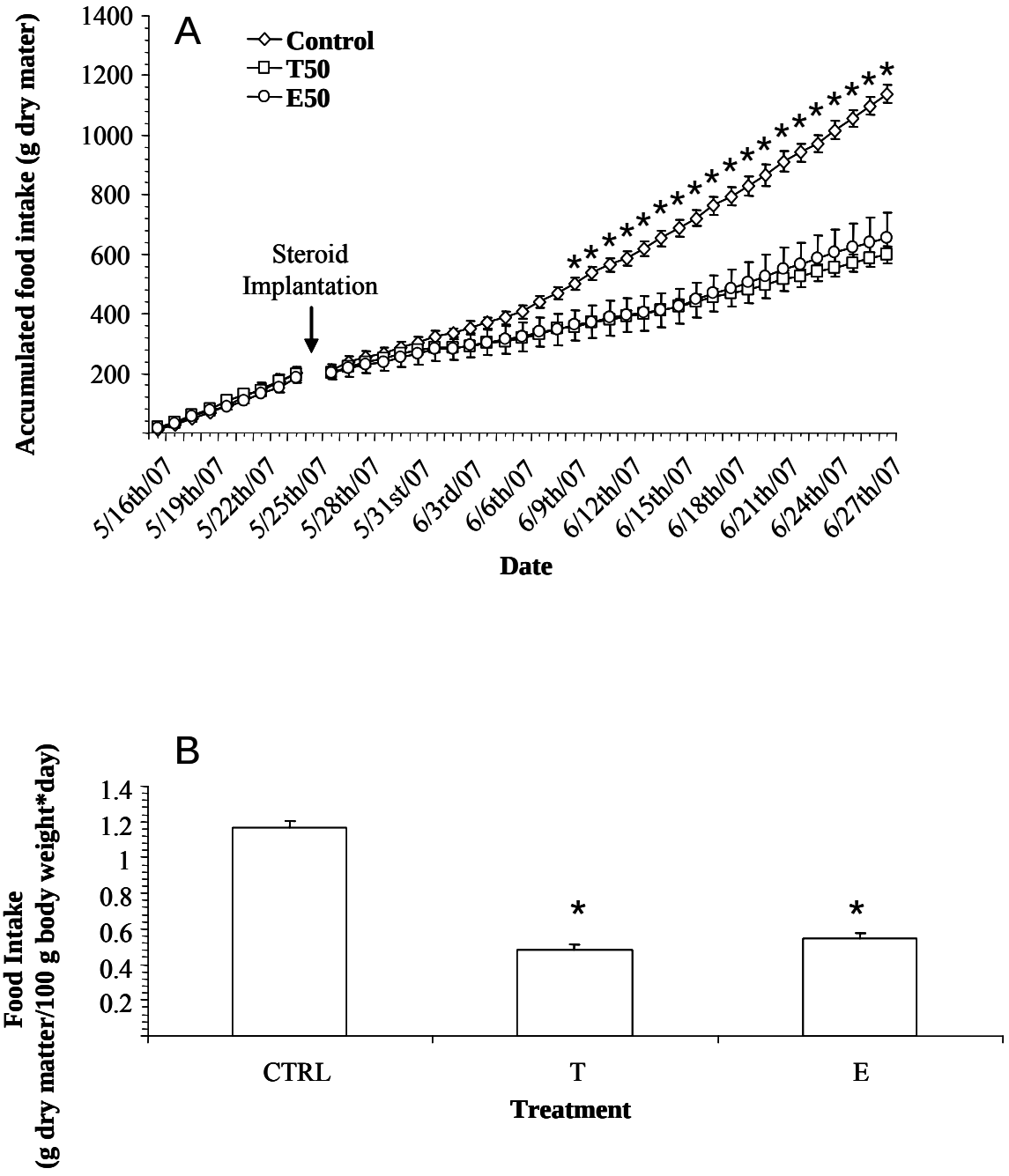
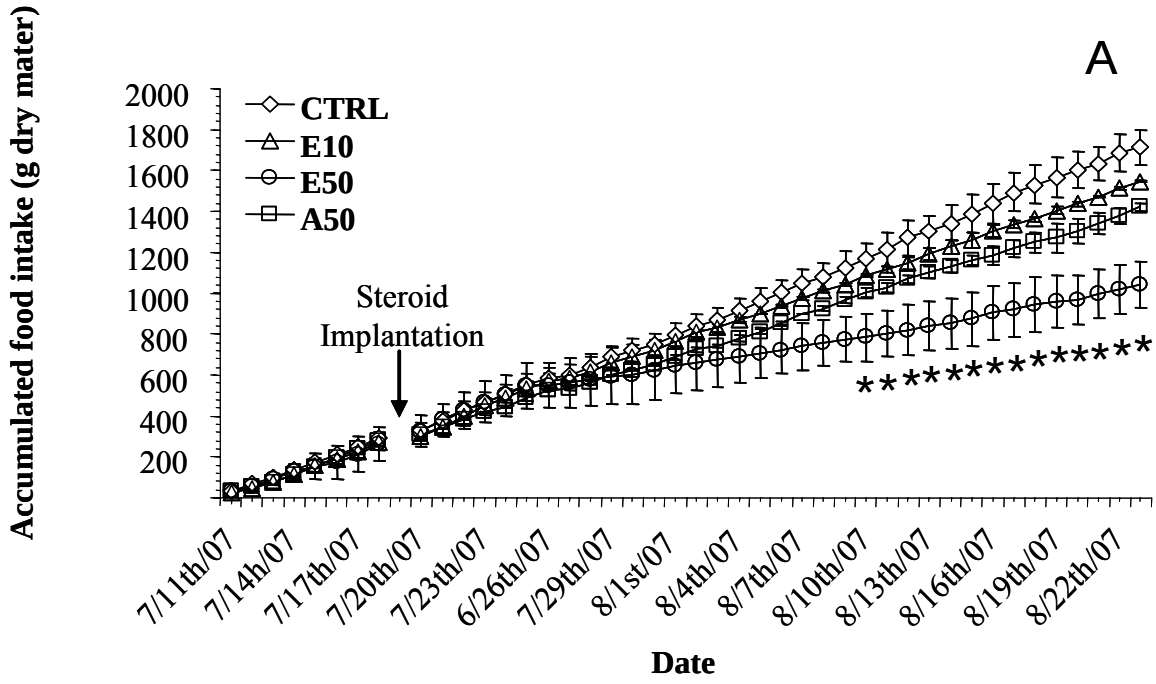

A
Control
T50
E50
Accumulated food intake (g dry mater)
1400
1200
1000
800
600
400
200
Steroid
Implantation
5/16th/07
5/19th/07
5/22th/07
5/25th/07
5/28th/07
5/31st/07
6/3rd/07
6/6th/07
6/9th/07
6/12th/07
6/15th/07
6/18th/07
6/21th/07
6/24th/07
6/27th/07
Date
B
Food Intake
(g dry matter/100 g body weight*day)
1.4
1.2
1
0.8
0.6
0.4
0.2
CTRL
T
E
Treatment

Figure 3


A
Accumulated food intake (g dry mater)
2000
1800
1600
1400
1200
1000
800
600
400
200
CTRL
E10
E50
A50
Steroid
Implantation
****
7/11th/07
7/14h/07
7/17th/07
7/20th/07
7/23th/07
6/26th/07
7/29th/07
8/1st/07
8/4th/07
8/7th/07
8/10th/07
8/13th/07
8/16th/07
8/19th/07
8/22th/07
Date

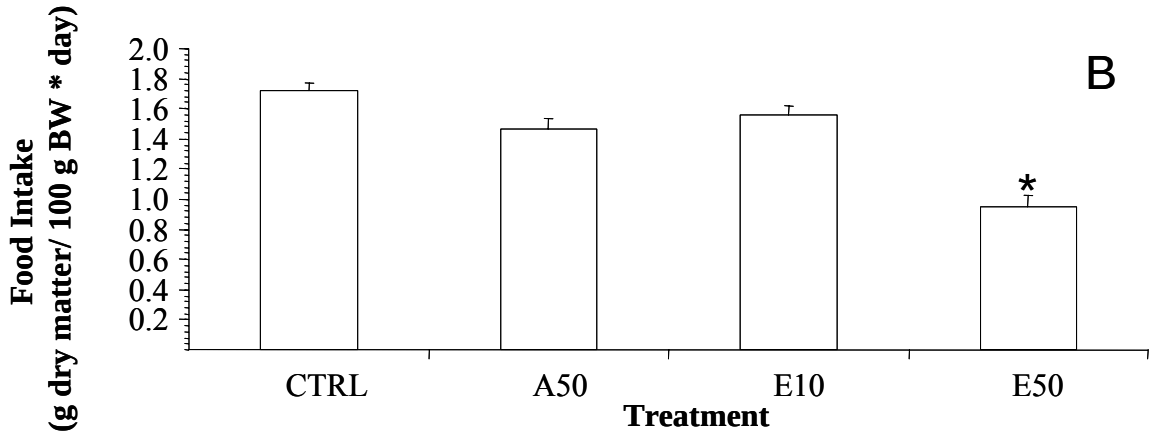

B
Food Intake
(g dry matter/ 100 g BW * day)
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
*
CTRL
A50
E10
E50
Treatment

Figure 4