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The influence of sex steroids in commercial fish meals and fish diets on plasma concentration of estrogens and vitellogenin in cultured Siberian sturgeon *Acipenser baeri*

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Abstract

Sex steroid levels were determined in various commercial fish meals and complete diets. The results obtained demonstrate that estrone (E_1), 17β-estradiol (E_2) and androgens (A) (testosterone and 11-ketotestosterone), are present in diets in high quantities (up to 935 ± 350 ng/100 g for E_2, 615 ± 190 ng/100 g for E_1 and $1\,100 \pm 120$ ng/100 g for A) and with considerable variation both between different diets, and between different batches of the same diet. These high levels of sex steroids are thought to influence the sex steroid plasmatic levels and plasma vitellogenin content in fish.

Plasma sex steroid assays in the Siberian sturgeon *Acipenser baeri* show that plasmatic 17β-estradiol levels are never significantly different between males and females at various stages of oogenesis and spermatogenesis, except in 6 year old females in stage IV, which corresponds to the maximum incorporation of vitellogenin. In males the plasma 17β-estradiol levels are exceptionally high and induce vitellogenin synthesis, as demonstrated by various chemical and immunological techniques.

Keywords : Sex steroids, fish diets, fish meals, vitellogenin, sturgeon.

Influence des stéroïdes sexuels contenus dans des farines de poissons et des aliments complets pour truite sur les niveaux plasmatiques d'œstrogènes et de vitellogénine chez l'esturgeon sibérien Acipenser baeri *élevé en pisciculture.*

Résumé

Les taux de stéroïdes sexuels ont été déterminés dans diverses farines de poisson et aliments complets utilisés en pisciculture. Les résultats obtenus montrent que ces farines et aliments contiennent de l'œstrone (E_1), du 17β-œstradiol (E_2) et des androgènes (A) (testostérone et 11-céto-testostérone) en quantités importantes (jusqu'à 935 ± 350 ng/100 g pour E_2, 615 ± 190 pour E_1 et $1\,100 \pm 120$ pour A) et variables, d'une farine ou d'un aliment à l'autre, et pour une même farine ou pour un même aliment, d'un lot à l'autre. Ces quantités importantes de stéroïdes sexuels apportées par l'alimentation pourraient avoir une influence sur les taux circulants de stéroïdes et de vitellogénine chez les poissons.

En effet, des dosages de stéroïdes sexuels plasmatiques réalisés sur des esturgeons sibériens *Acipenser baeri* à divers stades du développement des gonades montrent que les taux de 17β-œstradiol ne sont jamais significativement différents entre mâles et femelles sauf chez les animaux de 6 ans, lorsque les femelles sont au stade IV de l'ovogenèse c'est-à-dire lorsque l'incorporation de vitellogénine est maximale. Chez les mâles ces taux exceptionnellement élevés de 17β-œstradiol provoquent la synthèse hépatique de vitellogénine, détectée dans leur plasma par diverses techniques chimiques et immunologiques.

Mots-clés : Stéroïdes sexuels, aliments pour poisson, farines de poissons, vitellogénine, esturgeon.

INTRODUCTION

Sex steroids are principally synthetized by the gonads. In fish they are known to influence gonadal development (reviews by Hoar, 1969; Fostier *et al.*, 1983), morphological sexual characteristics (Yamamoto, 1969), somatic tissue anabolism and growth (Sower *et al.*, 1983). They also induce spermiation and ovulation at the end of gametogenesis (Fostier *et al.*, 1983).

In fish farming, accelerated growth and precocious sexual maturity (according to natural parameters) have often been reported (Saunders *et al.*, 1982 for trout or salmon, Sokolov *et al.*, 1986 and Williot *et al.*, 1988 *a*, *b* for Siberian sturgeon). This optimisation of physiological parameters can generally be related to exceptional temperature conditions, genetic selection or genetic manipulation and/or better feeding. To our knowledge, the only existing study on this topic is that of Sower and Iwamoto (1985) who identified testosterone in commercial fish diets and discussed the different ways in which this steroid could influence various physiological functions.

Preliminary data obtained on Siberian sturgeon have shown high levels of 17β-estradiol correlated with high levels of a female plasma protein (vitellogenin) in males as well as in females fed on a commercial trout diet (Pelissero and Le Menn, 1988 *b*; Pelissero *et al.*, 1988 *c*).

These results suggest a possible influence of diet on increased plasma sex steroid levels and consequently on vitellogenin content.

We thus tested different fish meals and complete diets to determine the levels of sex steroids. Fish diets are generally derived from mature teleost fish (whole fish or viscera) which often have high plasma levels of sex steroids.

MATERIAL AND METHODS

Fish

Siberian sturgeons, *Acipenser baeri*, were imported to France from the U.S.S.R. in 1974 and 1982 and reared at the INRA fish-farm CEMAGREF hatchery of Donzacq (Landes) at a constant temperature of 17°C. These particular conditions cause rapid growth and precocious sexual maturity. First reproduction occurred in 5-year-old males and 7-year-old females, twice as early as in the U.S.S.R. (Williot and Brun, 1982; Williot and Rouault, 1982; Akimova *et al.*, 1978). Fish were sexed by biopsy of the gonad followed by histology and light microscopy. The different stages of oogenesis were identified with a Jeol 100 S electron microscope at the Electron Microscopy Department of the University of Bordeaux-I. Reproduction has already been successfully carried out in France (Williot and Rouault, 1982; Williot and Brun, 1982) but every age-class was not available for experimentation. Thus for this study we used 15 males and 15 females of 4, 5, 6 and 10-year-old fish.

Diets

Six different fish meals (meal M_1 to M_6) from different origins (one Danish, one Islandic, two Chilean and two Norvegian), and three complete diets (diet A, B, C) were tested in this study. The fish meals were provided by Dr. S. Kaushik (Fish nutrition laboratory, INRA, Saint-Pée-sur-Nivelle, France), and fish diets from three different commercial producers were obtained from private trout farms. The diets and meals were stored for only one week in the laboratory before assay in order to test their normal conditions of use. The four different batches of diet B were obtained from two different fish farms and from four different deliveries of the same diet for spawners. All samples were treated in the laboratory within one week after their arrival.

The samples (1 g) were sonicated for 1 min in 5 ml of phosphate buffer saline used in radio immuno assay (RIA) (0.01 M phosphate buffer, 9% NaCl, pH 7.25) and then incubated for 2 hours with 2000 desintegration per minute of ^{3}H steroids (2, 4, 6, 7-^{3}H estradiol, 2, 4, 6, 7-^{3}H estrone and 1, 2, 6, 7-^{3}H testosterone) as internal standards to evaluate the extraction and chromatography ratio of collection (the value of extraction efficiency was generally between 55 and 65%). Extraction in cyclohexane-ethyl acetate solvent (1-1) was then performed twice at 20°C by a 5-minute-centrifugation (3000 g) by adding extraction solvent to meal in suspension in RIA phosphate buffer saline. The aqueous phase containing meal and non-extracted steroids remained in the tube and the organic phase containing extracted steroids and aromatic products was collected. Extracts were then evaporated and diluted in the chromatography solvent (dichloromethane-methanol, 95-5). The steroids assayed were separated on an LH 20 Sephadex column (8-0.5 cm) and the different fractions obtained were then evaporated and diluted in RIA phosphate buffer saline to perform the immunologic reaction. Assays were performed as described previously by Fostier and Breton (1975) with anti-estrone and anti-17β-estradiol from the Steranti Company Ltd. (St-Albans, England) and anti-androgens (anti-testosterone and anti-11 ketotestosterone) from Dr. Fostier (I.N.R.A., Rennes, France). ^{3}H labelled steroids were purchased from Amersham (Buckinghamshire, England) and unlabelled steroids from Steraloids Ltd (Croydon, England).

Plasma analysis

Blood samples were drawn from the caudal vein with heparinized seringes. Plasma was separated from

blood by a 5 min. centrifugation at 10000 g performed immediatly after the collection and then stored at 4°C until analysis. Plasma sex steroid contents were measured in the same way but without sonication or centrifugation before extraction.

Purified vitellogenin was obtained by double chromatography (Sepharose 6 B and DEAE Trysacryl M) as described earlier (Pélissero *et al.*, 1987; Pélissero, 1988 *a*). Chromatographic techniques were performed on plasma obtained from immature animals (1 kg) pretreated by 12 injections of 0.25 mg/kg 17 β-estradiol diluted in phosphate buffer saline (pH 7, 0.02 mM, 1 g/l gelatine) supplemented by 1/10 benzyl alcohol at two days intervals.

Yolk fraction was obtained by crushing fragments of ovary in phosphate buffer saline 0.1 M (pH 7.4, 9% NaCl). The extract was then centrifuged twice for 20 min. at 100000 g, 4°C and the middle phase collected and stored at −20°C.

The immunoprecipitation technique was performed according to Ouchterlony (1953), in 1% agarose gels in a veronal buffer (0.05 M, 1.5% HCl 1 N, pH 8.2) supplemented by 0.05% of sodium azide. The antiserum used was obtained against purified vitellogenin and its high specificity was tested on vitellogenic oocytes by an immunocytochemical method described previously (Pelissero *et al.*, 1988 *d*).

The alkali-labile phosphorus technique (Craik and Harvey, 1984) was used to evaluate plasma vitellogenin.

Polyacrylamide gel electrophoresis (PAGE) was performed in 4-16% acrylamide gradient gel with 3% stacking gel, in tris-glycine buffer (0.05-0.3 M, pH 8.3) under 200 V, for 6 hours. Volumes of samples were previously tested for each material and fixed to 1 µl of male plasma, 2 µl of female plasma, 0.25 µl of 17 β-estradiol treated immature fish plasma and 3 µl of a 5 mg/ml of purified vitellogenin solution diluted in phosphate buffer saline. Two types of staining were performed: Coomassie Brilliant Blue R 250 for protein according to the "Pharmacia" process (1980) and Sudan Black for lipoproteins (Prat *et al.*, 1969). Immunoblots on nitrocellulose sheets were performed with anti-purified vitellogenin immunserum at a concentration of 1/1 500 and under 0.8 mA by cm^2 for 2 hours.

RESULTS

Sex steroids in diets

The results obtained on the different fish meals and on the three complete diets showed relatively high amounts of sex steroids and considerable variations according to the diet (*table* 1) and from batch to batch of the same diet (*table* 2). Values for 17 β-estradiol varied between 40 ng/100 g (meal M_1) to 1510 ng/100 g (meal M_2). Androgen levels also showed a high variability (240 ng/100 g in M_2; 1340 ng/100 g in M_3). Estrone levels were less variable but just as high (270 ng/100 g in diet B; 940 ng/100 g in M_4). For meal M_4 and diet A, the general amount of sexual steroids was rather low, but in the other meals and diets tested, one or two sex steroids were present in large quantities. The results for different batches of the same diet present significant variations (from 47 ± 23 ng E_2/100 g to 650 ± 170 ng E_2/100 g and from 650 ± 120 ng A/100 g to 1430 ± 300 ng A/100 g) for diet B where the moderating value is the confidence interval obtained according to Student's *t* test (*i. e.* standard error multiplied by *t* (5%) value) which could mean that the diet formulae are not constant in each batch (*table* 2).

Table 1. — Sex steroid amounts (E_2: estradiol, E_1: estrone, A: androgens) in different complete diets (denoted A, B, C) and in six different fish meals (denoted M). Within parentheses is the confidence interval calculated from standard error multiplied by *t* value for 5% error.

Diet	Steroids		
	E_2 (ng/100 g)	E_1 (ng/100 g)	A (ng/100 g)
A	375 (±80)	430 (±75)	280 (±65)
B	240 (±60)	270 (±54)	1100 (±120)
C	615 (±190)	935 (±350)	1040 (±470)
M_1	40 (±9.5)	490 (±95)	520 (±40)
M_2	1510 (±310)	560 (±100)	240 (±90)
M_3	105 (±25)	910 (±165)	1340 (±180)
M_4	1490 (±330)	940 (±180)	875 (±240)
M_5	100 (±20)	350 (±60)	790 (±220)
M_6	1490 (±320)	520 (±95)	1330 (±415)

Table 2. — Sex steroid measurements in four different batches from the same complete diet denoted B. (E_2: estradiol; E_1: estrone; A: androgens). Values within parentheses are the confidence intervals for each concentration, calculated from the standard error multiplied by *t* value for 5% error.

Diet	Steroids		
	E_2 (ng/100 g)	E_1 (ng/100 g)	A (ng/100 g)
B	240 (±60)	270 (±54)	1100 (±120)
B_1	47 (±23)	520 (±250)	660 (±120)
B_2	53 (±15)	820 (±420)	650 (±120)
B_3	650 (±170)	670 (±80)	1430 (±300)

Sex steroids in plasma

The 17 β-estradiol plasma levels found in male and female Siberian sturgeons (*table* 3) were not significantly different in 4, 5 and 10-year-old fish. In 6 year-old fish (*i. e.* when the females are in the fourth stage of oogenesis) the 17 β-estradiol levels are significantly different from those of males according to a Student

Table 3. – Sex steroid levels (E_2: estradiol; E_1: estrone, A: androgens i.e. testosterone and 11 keto-testosterone) in plasma of Siberian sturgeon *Acipenser baeri* bred in fish farm. Within parentheses is confidence interval ($se \times t_5$).

	Animals	Steroids		
		E_2 (ng/ml)	E_1 (ng/ml)	A (ng/ml)
♀	(4-year-old fish)	16.6 (±1.34)	10.8 (±7.2)	5.8 (±0.5)
♂		14.4 (±0.95)	3.3 (±1.5)	104.4 (±47)
♀	(5-year-old fish)	12.9 (±4.3)	9.5 (±1.0)	4.6 (±1.0)
♂		11.5 (±2.8)	4.0 (±0.6)	115 (±40)
♀	(6 year-old fish)	18.1 (±9.3)	17.6 (±11.8)	33 (±14.2)
♂		5.6 (±2.0)	3.5 (±2.2)	82 (±6.5)
♀	(10 year-old fish)	19.3 (±2.7)	10.3 (±1.5)	109 (±25)
♂		26.7 (±4.7)	4.1 (±1.1)	178 (±6.5)

test (females: 18.1±9.3 ng/ml; males: 5.6±2 ng/ml). In 5-year-old fish, in the early vitellogenic stage, 17 β-estradiol levels were the same for both sexes (females: 12.9±4.3 ng/ml, males: 11.5±2.8 ng/ml). In 10-year-old fish the mean level of 17 β-estradiol was 26.7±4.7 ng/ml in males and 19.3±2.6 ng/ml in females. In this case several males had higher plasma 17 β-estradiol concentrations than any females.

Vitellogenin in plasma

For male plasma, yolk and vitellogenin, the immunoprecipitation technique gave a continuous arc between the three pits. Yolk gave an additional single over-all arc, extending from the purified vitellogenin to the male plasma pits (*fig.* 1).

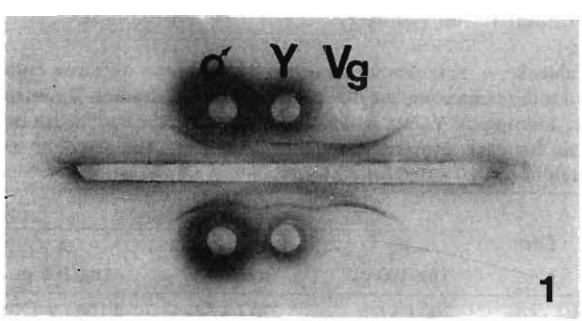

Figure 1. – Immunoprecipitation test performed with anti-purified vitellogenin immunoserum (at a concentration of 1/2). ♂ is male plasma (concentration 1/2); Y is yolk (concentration 1/2); Vg is purified vitellogenin at a concentration of 2.5 mg/ml in phosphate buffer saline.

The alcali-labile phosphorus technique indicated that there was no significant difference between the plasma alkali-labile phosphorylated protein levels of males and early vitellogenic females. Differences appeared in 6-year-old fish according to Student test when females reach the most active vitellogenin incorporation stage (*table* 4).

Table 4. – Plasma levels of vitellogenin measured by the alkali-labile phosphorus technique by reference to purified vitellogenin range, in Siberian sturgeon *Acipenser baeri* bred in fish farm. Within parentheses is the standard error multiplied by t_5 value.

Age	♂	♀
4- year-old fish (mg/ml)	2.1 (±0.6) end of spermatogenesis	2.1 (±0.4) non vitellogenic
5-year-old fish (mg/ml)	2.2 (±0.6) spermiation	2.5 (±0.3) beginning of vitellogenesis
6-year-old fish (mg/ml)	2.1 (±0.3) spermiation	6.8 (±1.2) vitellogenesis

Polyacrylamide gel electrophoresis stained by Coomassie Blue showed that males have a plasma protein exhibiting the same migration as vitellogenin. This protein, which has a high heterogeneity of electric charge in native gel, was also stained by the Sudan Black technique and presented a specific labelling in immunoblotting with an immunoserum obtained against purified vitellogenin (*fig.* 2).

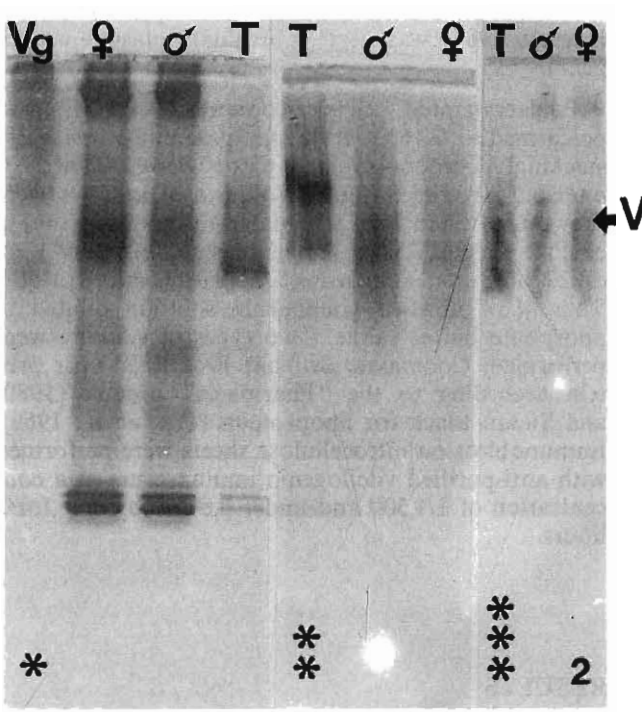

Figure 2. – Polyacrylamide gel electrophoresis: (*) stained with Coomassie Brilliant Blue R 250; (**) stained with Sudan Black; (***) revealed by immunoblotting with an immunoserum obtained against purified vitellogenin (at a concentration of 1/1 500). T is animal treated with 17 β-estradiol injections (1/4 µl); ♂ is male plasma (1 µl); ♀ is female plasma (2 µl); Vg is purified vitellogenin at a concentration of 5 mg/ml in phosphate buffer saline (3 µl).

DISCUSSION

Sex steroids in diets

The sex steroid levels found in the different fish meals and in the complete diets tested were relatively high although presenting considerable variations. These levels are in the range of the testosterone levels found in fish diets by Sower and Iwamoto (1985) (0.40 ng/g testosterone for the less concentrated and 7.0 ng/g testosterone for the more concentrated diet). Considering that the complete diet contained between 30 and 60% of fish meal (according to the specification provided by the feed manufacturers), the levels that we obtained for fish meals and complete diets are in accordance with these earlier results. However it must be noted that the extraction technique is based on the evaluation of an extraction efficiency ratio which assumes that the steroids of the sample reacts exactly like the added radioactive steroid. If this is not the case the evaluated extraction efficiency ratio obtained by this technique is higher than in reality, and results in an under-estimation of the true sex steroid concentration.

In addition to 30 to 60% of fish meal (the particular formula of each brand) the complete diets usually contain other ingredients like meat meal, soya-bean oil, soya-bean extracts, wheat germ, wheat extracts, alfa meal, yeast, mineral compounds and vitamins with any supplementation of sex steroids. Then it is reasonable to assume that the main potential source of sex steroid must be fish meals since they are the main component by weight in the complete diets.

Our results indicate that sex steroid levels in the same complete diet can vary from batch to batch. These variations could not be controlled since the fish species used for fish meals are not the same for all batches of the same meal. The composition of fish meal depends generally on the availability of species at the time of capture by fish meal producers. Generally one main species is used, for example anchovies for Chilian meals, poor-cod for Norwegian meals, and cod for Icelandic meals. Sex steroid levels in complete diets depend on: (i) the sexual stage of the fish used to make the fish meals; (ii) the species used to make the meal; (iii) the type of meal used according to availability or cost to make the complete diet. Generally, the choice of fish meal for a commercial diet is based on the proximate composition (protein, fat, amino-acid contents) and no attention is given to the steroid contents (Kaushik, pers. comm.). Our results on the steroid levels of meals suggest that the quality of fish meals can vary considerably from this point of view. The method of preparing the diet could also influence the sex steroid levels. Several different methods are used for the preparation of fish meals before and after cooking between 70 to 160°C in a moist atmosphere: for instance vacuum drying, flame drying under 70°C and, in particular conditions, formic and perchloric acid treatments or extrusion at 120-130°C under 3×10^5 Pa pressure for a few seconds. This last treatment has the highest temperature level for preparation of complete diets. Since sex steroids are usually considered stable up to 250°C (fusion temperature), none of these treatments would be expected to eliminate them completely from the final product, although chemical transformation of the molecules (*e.g.* by oxydation) must be considered. The three complete diets tested (A, B and C) were made specially for fresh water spawners and although commercial secrecy prevents verification of this fact, it would seem that all fresh water spawner complete diets are obtained by simple vacuum drying without any high temperature treatment ($t < 70$°C). This would explain the large variations in sexual steroid levels found in different fish meals or diets, for example particularly high amounts of androgens in diet B and C (*table* 1). This variability results directly from the different source of fish used to make meals and diets.

Sex steroids in plasma

In sturgeon plasma the 17 β-estradiol levels in males are relatively high since they are never significantly different from those of females in stage III of oogenesis (early vitellogenin incorporation in oocytes). If these 17 β-estradiol levels induce vitellogenin hepatic secretion and oocyte incorporation in females, they must also provoke vitellogenin secretion in males (Pelissero, 1988 *a*).

Vitellogenin in plasma

The results obtained by the alkali-labile phosphorus technique show that male sturgeon plasma contains a level of phosphorylated alkali-labile protein which is never significantly different from that of early vitellogenic females according to Student test. This result is in accordance with the plasma estradiol measurements but is at variance with those previously obtained by other authors. Usually, the phosphorylated alkali-labile proteins in fish plasma are considered to be vitellogenin and, as such, specific to females (Wallace and Jared, 1968; Craik, 1978; Nagler *et al.*, 1987).

Polyacrylamide gel electrophoresis (PAGE) of male plasma revealed a protein with the same migration as vitellogenin. In native gel this protein presents a high electric charge heterogeneity since the bands obtained are fairly broad. It also contains lipid fractions such as vitellogenin and cross-reacts with the immunoserum obtained against purified vitellogenin. The immunoprecipitation technique used here shows that males have a plasma protein which presents a common antigenic conformation with yolk and purified vitellogenin. These results indicate the yolk nature of the lipidic, phosphorylated and alkali-labile protein found in male sturgeon plasma and identifies this protein as vitellogenin.

To our knowledge, very few studies have been performed on sturgeon steroid. The first one dealt

with corticosteroids and testosterone assays performed on two males *Acipenser oxyrhynchus* (Sangalang *et al.*, 1971), the second was performed on *Acipenser transmontanus* used for artificial reproduction by Lutes and coworkers (Lutes *et al.*, 1987). In this study only progesterone, corticosteroids and 17 α, 20 β-dihydroxy-4-pregnen-3-one were measured. Thus, there are as yet no data on natural sex steroid cycles in wild sturgeons in general, and no data at all concerning *Acipenser baeri*. The lack of published data on sex steroid and vitellogenin assays in sturgeons leads us to compare our results with those of teleost fish (Fostier *et al.*, 1983) or the quite similar ones of non teleost oviparous fish (Craik, 1978; Garnier *et al.*, 1988).

In this context the estradiol levels in males responsible for the presence of vitellogenin could be considered abnormally high. We suggest that it could be related to diet contamination, especially if one considers the duration and the periodicity of estradiol administration. In a previous paper, we observed that a change from diet A to diet B induced a drop in the 17 β-estradiol and vitellogenin levels in the plasma of male sturgeon (Pelissero *et al.*, 1988 *c*).

If diet could be a factor in the case of sturgeon, it could obviously also be so in other artificially-reared fish. The literature has until now tended to consider vitellogenin as an exclusively female plasma protein and as such used to identify sex.

(i) Several studies have related the phosphorus alkali-labile content in plasma to vitellogenin content and used it as a specific parameter for identifying females (Wallance and Jared, 1968; Craik, 1978; Craik and Harvey, 1984; Nagler *et al.*, 1987).

(ii) The immunoprecipitation tests have previously been successfully used with artificially-reared fish to distinguish females from males (Le Bail and Breton, 1981). This has been confined to fish during advanced vitellogenesis, and could be explained by its low sensitivity which does not allow identification of low concentrations of vitellogenin possibly present in male plasma.

For several years, more sensitive methods such as RIA or enzyme-linked immunosorbent assay (ELISA) techniques which allow the detection of rising levels of vitellogenin at the onset of vitellogenesis, have provided the best approach. ELISA tests did not identify vitellogenin in wild male soles (Nunez Rodriguez *et al.*, 1988; Nunez Rodriguez *et al.*, 1989). RIA techniques performed on farmed fish species such as rainbow trout (Copeland *et al.*, 1986) or coho salmon and various salmonid species (Benfey *et al.*, 1987) allow identification of low levels of vitellogenin in immature males and females of the homologous species, in accordance with our findings on the sturgeon. The difference between sturgeon and other teleost fish is the presence of high levels of vitellogenin in male and immature sturgeon plasma detectable by methods of low sensitivity. There are two possible explanations for this phenomenon: higher sensitivity of estrogen receptors by liver in sturgeon, which could initiate vitellogenin secretion with lower estrogen plasmatic levels, or a slower metabolism of ingested steroids. These two hypotheses could be supported by the fact that wild sturgeon are not piscivorous under natural conditions (Ruban and Sokolov, 1986; Sokolov *et al.*, 1986). Instead, they eat insects and worms which are known to have ecdisteroids different from fish steroids. Therefore trout diets containing fish meals seem unsuitable for sturgeon.

Further experimentation will be necessary to test these hypotheses.

Despite of the small amounts of sex steroids ingested daily we suggest that they could have a physiological impact on reared sturgeon. According to the literature, the main effects of a long term administration of sex steroids on young fish are: an accelerated growth linked to the anabolic effect of steroid (Fagerlund and McBride, 1975; Donaldson *et al.*, 1978), sexual precocity (Sower *et al.*, 1983), modification of the smoltification process (Sower and Iwamoto, 1985), appearance of intersex animals (Yamazaki, 1972; Tayamen and Sheldon, 1978, Sower *et al.*, 1983; Billard, 1983) and probably, a failure to produce a sterile population since sex steroids orally administrated are known to have an effect on gonadal differentiation (Goetz *et al.*, 1979; Solar *et al.*, 1984; McGeachin *et al.*, 1987). Some pathological effect of orally-administered estradiol have also been demonstrated by Herman and Kincaid (1988) in young rainbow trout. Principal symptoms of this pathology are kidney degeneration and hyperplasic structure in the liver. Moreover Kaushik *et al.* (1988) found that in sturgeon some liver damage occurred with high levels of digestible carbohydrates. In sturgeon it is interesting to note that M_3, which was the meal presenting the highest levels of sexual steroids, was shown to promote excellent growth of yearling sturgeons (Kaushik, pers. comm.).

In female spawners in several species it has been demonstrated that a drop of plasma 17 β-estradiol level occurs prior to maturation and ovulation (Fostier *et al.*, 1983) which would correspond to a negative feed back of estradiol on GTH secretion prior to ovulation. Therefore, if in the sturgeon an external source of 17 β-estradiol is constantly maintained, this could have an inhibitory effect on maturation and ovulation.

CONCLUSION

It seems that sexual steroids introduced by artificial feeding in fish farms might pose a problem, especially considering the sexual steroid metabolism rate in fish farm species. This metabolism may vary considerably with the temperature from one species to another and for the same species from one gametogenesis stage to another (Querrat *et al.*, 1985; and Baroiller *et al.*, 1987). Moreover, these variations may be further

increased because the sex steroid levels in fish meals and in complete diets vary considerably between meals and diets and also, between batches of the same diet.

In sturgeon, this dietary steroid intake appears to be responsible for high plasma estradiol levels in males and consequently for the presence of vitellogenin in their plasma. However, since no data is as yet available on sex steroid levels in wild Siberian sturgeon populations, we cannot be sure whether or not these findings are normal. Indeed several studies show a possible action on growth, health and reproduction. Such effects on sturgeon must be investigated.

Even trough sex steroid levels in commercial diets are generally lower than those currently use in experiments to determine their effects on fish, their presence must be carefully assessed prior to experiments or particular treatments in fish farms.

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