Feeding effect of cholesterol in the diet on sex hormones concentrations and the gonads' growth of yearling common carp (*Cyprinus carpio*)

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Abstract:

BACKGROUNDS: There are some evidences that cholesterol can affect fish reproductive system. **OBJECTIVES:** In this study the effect of high diet cholesterol on fish sex hormones and gonads weight was investigated. **METHODS:** A randomized experiment was conducted on 90 immature common carp. The fishes were divided into three groups with three replicates each (control, 0.5% cholesterol diet, and 1% cholesterol diet). After one-month feeding, progesterone, estrogen and testosterone were measured. **RESULTS:** The level of progesterone in control group, 0.5% cholesterol and 1% cholesterol diet were 1.83±0.42, 2.45±0.38 and 2.62±0.52 ng/ml, respectively. Also the level of estrogen in control group, 0.5 % and 1% cholesterol diet were 3670.34±186.26, 3791.20±98.48, and 3836.78±81.74 pg/ml, respectively., while those of testosterone levels were 1.75±0.319, 2.09±0.425, and 2.25±0.321 ng/ml, respectively. The highest and the lowest body weight and gonad weight were observed in fish fed %1 cholesterol and the control, respectively. The results showed positive effects of cholesterol on sexual hormones, the 0.5 % cholesterol diet and the 1% cholesterol diet did not show any significant difference in the sexual hormones levels. **CONCLUSIONS:** The results indicated that the effect of cholesterol on the these sex hormones, gonad weight, body length and body weight was significant ($p<0.01$). We can conclude that diets of 0.5% cholesterol can be used to increase the amount of sexual hormones in common carp.

Introduction

The production of common carp and Chinese carp is the largest form of aquaculture in Iran. Although we do not have access to recent and precise production figures, because they are not published, Common carp and three species of Chinese carp, are almost the only kinds of aquaculture system in the Khuzestan province. Cholesterol is a naturally occurring steroid which is mainly produced by animal cells. There is some evidence that cholesterol can affect a fish's reproductive system (Diazfontdevila,and Bustosobregon, 1993; Bjerkeng et al., 1999; Montoudis et al., 2004; Leroy et al., 2010). Much research has been undertaken to control and manage the reproduction of cultured species by means of exogenous steroid, or non-steroids, administered by immersion or in the diet at various stages of development (Burzawa-Gerard, and Dumas-Vidal, 1991; Carwell and Liley and Kroon, 1995; Christians, J.K.; Williams, T.D.

1999; Bennetau-Pelissero et al., 2001; Engelhardt, 2004). Although a few studies have involved cholesterol as a sex hormone precursor (Rainie et al., 2006), there is no study on the effect of cholesterol on steroid production in common carp.

Sex hormones are steroids secreted from the testes and the adrenal glands in both male and female animals. As in other vertebrates, cholesterol acts as precursor for the biosynthesis of sex steroids in fish. Steroid biosynthesis mainly occurs in the somatic cells of the testis and ovary, which results in the production of an array of sex steroids (Kumakura et al., 2004). The pathway initiates with the synthesis of the steroid precursor pregnenolone, via cleavage of cholesterol, is followed by the production of progesterone. In the ovary, thecal steroids (mainly testosterone), are made into granulosa cells where the enzyme aromatase is expressed, resulting in the conversion of testosterone to estradiol-17h. The latter steroid is necessary for oocyte growth (Devlin and Nagahama 2002).

Sex steroids are not only essential for bone growth, but also for maintenance of skeletal integrity as shown by skeletal changes following sex steroid deficiency in humans and rodents. Cholesterol may appear to be involved in the feedback control of sex hormone levels in fish. Some experiments showed that adding cholesterol to the food of females reversibly reduces circulating estradiol, or may play a role in increasing their hormone production by stimulating and modifying steroid levels (Vandenput et al., 2004, Riggs et al., 2002, Callewaert et al., 2009). Therefore in an initial approach,we investigated sex hormone (testosterone, progesterone and estrogen) levels in the serum of common carp as a fish model. The factor condition, or length-weight relationship of an individual fish, can be another important response to hormones. This raises the possibility that this effect may be found due to the impact of sex hormones on the body metabolism of the fish. In this study, we, also, investigated the influence of cholesterol on a fishes' gonad and body weight.

Materials and Methods

Experimental groups: Ninety common carp, weighing 65±5.1 g, were obtained from a fish farm in Khuzestan Province, Iran. The fish were divided into three groups (control, 0.5% cholesterol and 1% cholesterol) with three replicates for each group (n=10). All fish were fed 3% body weight, daily. Two groups were fed a high-cholesterol diet (0.5% cholesterol and 1% cholesterol added to commercial fish food) for 4 weeks, and one group receiving only commercial diet (control group, under 0.01% cholesterol). The water quality consisted of temperature 22 °C, dissolved oxygen 6-7 mg/l, pH 7.6 and salinity 1.3 ppt.

Blood sampling: After a one-month period, blood samples were collected from fish via the caudal vein. After clotting (in refrigerator, 4°C), blood samples were centrifuged (2000g for 5 minutes) and the obtained serua were stored at -20°C until hormone assay. All 30 fish in each group were sampled individually.

Biometry and gonad weight measurement: After blood sampling, the fish were euthanized and body length and total weight, gonad weight and the sex of fish, were determined.

Radioimmunoassay: Progesterone, estrogen and testosterone were measured by radioimmunoassay (RIA) technique and the concentrations of these sex steroids in the fish serum samples were determined as described by Shimizu et al., 1985. Hormone kits (Biosource Europe S. A., Belgium) and gamma counter (Gamma-1, LTiGenesys) were used for the measurements. All samples were measured in duplicate.

Statistical analysis: Data were analized using analysis of variance (PROC GLM) in the SAS statistical program and LSD test at probability level of 5%. The Pearson Correlation Test was used for correlation testing between parameters (weight and hormone concentrations). Results with a $p<0.05$, were regarded as significant.

Results

The levels of progesterone were 1.83±0.42; 2.45±0.38; and, 2.62±0.52 ng/ml, in the control group, the 0.5% cholesterol and 1% cholesterol, respectively. The level of estrogen in control group, 0.5% and 1% cholesterol diet were, 3670.34±186; 26,3791.20±98.48; and, 3836.78±81.74 pg/ml respectively. For testosterone these levels were, 1.75±0.355; 2.09±0.425; and, 2.25±0.321 ng/ml

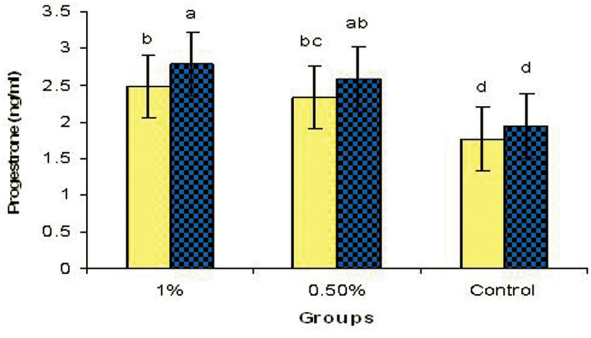

Figure 1. Comparison of progestrone levels (Mean±SEM) between male and female fish in the studied groups.* a, b, c, d- different letter in each bar means significant difference between parameters. Male Female

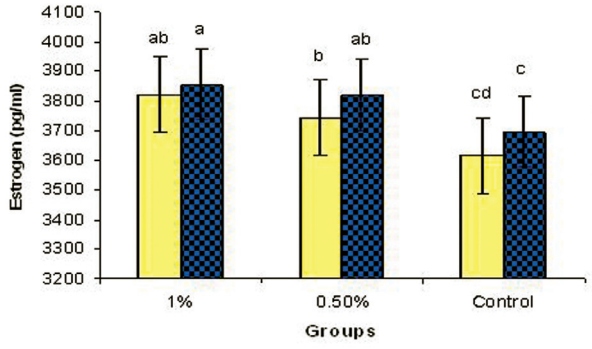

Figure 2. Comparison of estrogen levels (Mean±SEM) between male and female fish in the studied groups.* a, b, c, d- different letter in each bar means significant difference between parameters. Male Female

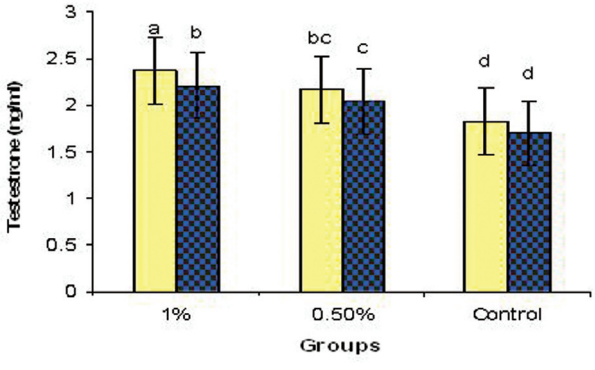

Figure 3. Comparison of testosterone levels (Mean±SEM) between male and female fish in the studied groups.* a, b, c, d- different letter in each bar means significant difference between parameters. Male Female

respectively. The results indicated that the effect of cholesterol on the sex hormones (estrogen, progesterone and testosterone), gonad weight, length, and body weight was significant ($p<0.01$). No significent difference was found in the contents of testestrone and progestrone between 0.5% and 1% cholesterol groups ($p>0.05$).

The mean levels of estrogen and progesterone in females were significantly higher than in the males in all groups. However, the mean level of testosterone in all males groups found to be significantly higher than in the females ($p<0.05$) (Figure 1, 2 and 3).

Correlation analyses revealed a significant positive correlation ($r =0.623$) between body weight and gonad weight in all groups (Table2).Furthermore, gonad weight showed a significant positive correlation with hormones level in both cholesterol groups as well as in the control group. No correlation was found between estrogen and progesterone level in the groups that were studied.

Discussion

There have been many investigations on sex manipulation in fish, using exogenous steroids (Piferrer, 2001; Devlin and Nagahama, 2002). The vast majority of this research has been undertaken to control the reproduction of cultured species, although a few studies have also explored the mechanisms of exogenous steroid action some experiments have involved in different androgens,estrogens,or precursor steroids administered by immersion or in the diet at various stages of development (Wonnacott et al., 2010). Different dosages and durations of treatment have been employed and summarized by Devlin and Nagahama 2002.

The results of these studies indicated that there is a significant effect from cholesterol on the sex hormones of estrogen, progesterone and testosterone. The mechanisms for raising hormone levels may be due to raising its precursor in the blood. The extra cholesterol level in food may act as precursor for the biosynthesis of sex steroids. Some experiments showed that adding it to the food for females, reversibly reduces circulating estradiol, or may play a role in increasing hormone production by stimulating

Table 1. Hormone levels, body length and gonad weight (Mean±S.E.M) of control and experimental groups in common carp in both sexes (number of fish in each group: n=10 with 3 replicate). * a, b, c- Different letters in each column show significant difference between parameters.

Group	Body length (cm)	Body weight (g)	Gonad weight (g)	Estrogen (ng/ml)	Testestron (pg/ml)	Progestron (pg/ml)
Control	19.52±1.062 b*	95.09±11.64 a	1.58±0.486 b*	3670/34±186.26 b*	1.75±0.319 b*	1.83±0.42 b*
0.5 % cholestrole	19.92±0.998 b	105.05±11.24 b	2.28±0.622 a	3791.20±98.48 a	2.09±0.425 a	2.45±0.38 a
1 % cholestrole	20.63±0.835 a	117.75±8.98 c	2.39±0.584 a	3836.78±81.74 a	2.25±0.321 a	2.62±0.52 a

Table 2. Summery of correlation between hormonal level and some of the body characteristics of common carp in all groups of the data pool. The figures show the r values between studied parameters in each column and raw values. *- correlated in 90% confidence (Significant) **- correlated in 95% confidence (Significant)***- correlated in 99% confidence, (Significant)ns- Non-correlated significantly (non Significant).

	Body weight	Gonad weight	Body length	Testosterone	Estrogen	Progesterone
Body weight (gr)	1	0.623***	0.465***	0.609***	0.567***	0.396***
Gonad weight (gr)		1	0.316**	0.581***	0.237*	0.555***
Body length (cm)			1	0.282**	0.402***	0.297**
Testosterone (ng/ml)				1	0.250*	0.457***
Estrogen (pg/ml)					1	0.214ns
Progestron (ng/ml)						1

and modifying steroid levels (Riggs et al., 2002, Callewaert et al., 2009). However, without doing repeated experiments and to gain knowledge of the specific mechanisms, it is difficult to know whether the changing of steroid biosynthesis will occur by administrating these steroids, or their precursors.

Both female, and male, carp, responded to the higher levels of cholesterol via increasing in the estrogen, progesterone and testosterone levels. As expected, the mean testosterone hormone in the females was less than that found in the males. This result indicates that common carp can respond to the high cholesterol diet by at least increasing androgen secretion, or decreasing androgen clearance. Testosterone, secreted by Leydig cells in the testis, is the primary steroid hormone that maintains male fertility. Serum testosterone levels might be a good marker of the Leydig cell function. Cholesterol is a typical food ingredient that can be used as a processor for this hormone and for increasing plasma levels of testosterone.

According to our results, it becomes evident that, due to the elevation in serum hormones during exposure to the high cholesterol diet, there was a significant effect on body and gonad weight (Table 1). We found that the maximum and minimum body and gonad weight was observed as a result of the use and lack of cholesterol, respectively. The level of sex hormone in Zebra fish strongly affected the embryonic offspring survival. This might be related to sex-specific effects of the hormone on egg weight, offspring development, and parental behavior (Engelhardt et al., 2004). Estradiol can stimulate the liver to produce albumin and yolk precursors and affect egg composition (Christians and Williams 1999). Albumen enhances embryonic protein synthesis and structural growth of the embryo (Muramatsu et al., 1990).

Although the comparison of the mean results suggests a positive effect of cholesterol on the sex hormones (estrogen, progesterone and testosterone), no significant difference was seem in the sexual hormone level. Therefore, diet concluding 0.5 % cholesterol can increase the levels of sexual hormones in common carp. The Mukhi et al., 2007 observations suggested an important role for thyroxin in the process of gonad sex differentiation and reproductive function. Thyroid function may be affected by cholesterol, directly or indirectly, due to sex hormone changes. Changes in steroid biosynthesis

during maturation in females are mediated in part by a reduction in the amount of ovarian aromatase enzyme,and consequently the reduced conversion of testosterone to estradiol in the follicle (Young et al.,1983). Kubokawa et al. (1999) reported salmon, the mean initial testosterone level in females (159.3± 66.0 ng/ml) was significantly higher than that in the males (34.9±6.2 ng/ml).

Although our study showed that application of steroids' precursors may influence the course of gonadal development. Sex receptors were identified in the early stages of a fish's gonads' development (Chang et al.,1999). Therefore, steroids can have an effect in early stages of the growth of fish and on gonad activity (Dulka et al.,1987). If sufficient levels of sex steroids are provided, particularly at stages of gonad development, early maturity may occur.The present study demonstrated a significant effect of 0.5% and 1% cholesterol diet over sex hormone level and gonad weight. The increasing of gonad weight is an interesting finding in the context of maturity considerations.

Cholesterol supplementation can be suggested as a promising method to use for brood stock feeding, because it seems to accelerate growth in fish, and avoids the undesired effects of hormone therapy. On the other hand, the cholesterol treatment, by changing the hormonal status, may induce vitellogenesis, oocyte maturation and eventually ovarian development. The recent report by Horner 2009, concludes that follicle estrogen can regulate bone mass. In the present study using younger juvenile carp (one year old) we were notable to show that they were receptive and able to induce maturation via cholesterol overfeeding. Our study showed that during sex maturation a little shift in steroid biosynthetic activity occurred in both sexes, which may have had effects on both somatic (body weight) and gonad tissues (gonad weight).

There are no reports on the normal values of sex hormones of common carp and other cyprinid species. Unfortunately, most of the biological and physiological studies on these topics have been done on other fish species (Kubokawa et al.,1999). The environmental conditions in the Khuzestan province are different from other regions. In this area, the fish growth rate is high and the fish mature sooner than expected period. Therefore, this study of sex hormones changes is the first for this fish species in this region.

In conclusion, cholesterol overfeeding is associated with high serum sex hormone concentrations. This treatment seems to induce changes in reproductive endocrine functions in maturing common carp. Therefore, we can reasonably assume that cholesterol overfeeding, for 1 month can result in maturity inducing effects in this fish species.

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مجله طب دامی ایران، ۱۳۹۱، دوره ۶، شماره ۱، ۲۸-۲۳

مطالعه تأثیر مقادیر مختلف کلسترول جیره بر میزان هورمون‌های جنسی سرم و رشد گنادها در ماهی کپور معمولی

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چکیده

زمینه مطالعه: کلسترول جیره می تواند به عنوان یک عامل موثر بر رشد گناد و هورمون های جنسی ماهی کپور معمولی باشد. **هدف:** هدف از این تحقیق بررسی تأثیرات کلسترول بر میزان هورمون های جنسی (استروژن، تستوسترون، پروژسترون) بوده است. **روش کار:** به این منظور ۹۰ قطعه ماهی کپور معمولی نابالغ به صورت کاملاً تصادفی به ۹ آکواریوم منتقل گردیدند. ماهی ها به سه تیمار هرکدام با ۳ تکرار شامل جیره ۱٪ کلسترول، جیره ۰/۵٪ کلسترول و شاهد (کمتر از ۰/۰۱٪ کلسترول) تقسیم شدند. پس از گذشت یک ماه میزان هورمون های استروئیدی اندازه گیری گردید. **نتایج:** میزان پروژسترون در گروه شاهد و گروه کلسترول ۰/۵٪ و ۱٪ به ترتیب ۰/۴۴ ± ۱/۸۳، ۰/۴۴ ±۲/۴۵ و ۰/۴۴ ±۲/۶۲ نانوگرم بدست آمد. این مقادیر برای استروژن ۱۲۲/۱۶ ± ۳۶۷۰/۳۴، ۱۲۲/۱۶ ± ۳۷۹۱/۲۰ و ۱۲۲/۱۶ ± ۳۸۳۶/۷۸ پیکوگرم و تستوسترون به ترتیب ۰/۳۵۵ ±۱/۷۵، ۰/۳۵۵ ± ۲/۰۹ و ۰/۳۵۵ ±۲/۲۵ نانوگرم بوده است. مقایسه میانگین ها نشان داد که بیشترین میزان وزن ماهی در گروه های با مصرف کلسترول ۰/۵ و ۱٪ کلسترول بوده و کمترین آن در گروه شاهد بوده است. **نتیجه گیری نهایی:** نتایج نشان دهنده اثرات معنی دار کلسترول بر میزان هورمون های جنسی (استروژن، تستوسترون و پروژسترون)، وزن گناد، طول و وزن ماهی می باشد که این اثر بین دو غلظت کلسترول معنی دار نبوده است. به طور کلی نتایج این تحقیق نشان داد که جهت افزایش میزان هورمون های جنسی در ماهی کپور معمولی می توان از روش اضافه کردن میزان ۰/۵٪ کلسترول به جیره استفاده نمود.

واژه‌های کلیدی: کلسترول، ماهی کپور معمولی، هورمون های جنسی، سرم، رشد گناد.

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