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Effects of Long-Term Feeding of Cottonseed Meal on Growth, Testis Development, and Sperm Motility of Male Channel Catfish *Ictalurus punctatus* Broodfish[1]

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Abstract

The effects of long-term feeding of cottonseed meal on growth, testis development, and sperm motility of male channel catfish *Ictalurus punctatus* were studied. Brood-sized male channel catfish were stocked into 0.04-ha earthen ponds in April 1992 at the rate of 120 fish per pond. The fish were fed a diet (32% protein floating catfish feed) containing either 0, 25, 37.5, or 52% cottonseed meal to satiation daily, except for winter months when fish were fed on days when the water temperature was 15 C or above. Fish were harvested in July 1994. Feed consumption was similar for fish regardless of dietary treatment. Feed conversion was higher for fish fed the two highest levels of cottonseed meal and weight gain was depressed in these groups. Testis weight, gonosomatic index, and sperm motility were not negatively affected by high levels of dietary cottonseed meal indicating that reproductive capacity was not diminished. Thus it would appear that up to 52% cottonseed meal could be used in the diets of brood-sized channel catfish unless maximum gain is important. In a practical situation, growth rates of brood-sized channel catfish are not as important as reproductive performance.

The nutritional value of cottonseed meal for channel catfish *Ictalurus punctatus* and its use in channel catfish feeds have been well documented (Dorsa et al. 1982; Robinson and Rawles 1983; Robinson et al. 1983; Robinson and Brent 1989; Robinson 1991; Li and Robinson 1993; Robinson and Li 1993; Robinson and Li 1994). Even though high levels of cottonseed meal can be used for grow out of catfish, the maximum level recommended for use in commercial catfish feeds has been limited to about 30% in part because of the lack of information on the effects of gossypol (a naturally occurring chemical found in cottonseed meal) on spermatogenesis of male fish (Li and Robinson 1993). Gossypol has been shown to inhibit spermatogenesis in humans and certain other mammals (Wu 1989). The present study was designed to test the effects of long-term feeding of cottonseed meal on growth and reproductive status of male channel catfish.

Materials and Methods

Four practical-type extruded feeds (Table 1) were formulated to contain 32% protein and meet all known nutritional requirements of channel catfish (Robinson 1989; National Research Council 1993). Although the nutritional requirements for channel catfish broodfish are not known, a typical growout feed has generally been assumed to provide adequate nutrition for broodfish. Dietary treatments were based on the level of cottonseed meal used and were as follows: diet 1, 0% cottonseed meal; diet 2,

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TABLE 1. *Percentage ingredient composition of experimental feeds (as fed).*

Ingredient	Feed number 1	2	3	4
Soybean meal, 48%[a]	42	21.25	15.35	—
Cottonseed meal, 41%[a]	—	25	37.5	52
Corn grain	27.26	20.46	13.31	16.04
Wheat middlings	20	22.5	22.5	20
Meat and bone/blood meal, 65%[a]	4	4	4	4
Menhaden fish meal, 61%[a]	4	4	4	4
Catfish oil	1.5	1.5	2.5	2.5
Defluorinated calcium phosphate	1	0.75	0.63	0.5
Vitamin C-free premix[b]	0.1	0.1	0.1	0.1
Vitamin C[c]	0.065	0.065	0.065	0.065
Mineral premix[d]	0.025	0.025	0.025	0.025
L-lysine[e]	—	0.35	0.55	0.76
Free gossypol (mg/kg)[f]	0	200	300	400

[a] Protein level of meal.

[b] Vitamins supplied (mg/kg diet): A, 4400; D_3, 2200; E, 66; B_{12}, 0.05; riboflavin, 13.2; niacin, 88; pantothenic acid, 35; menadione bisulfite, 4.4; folic acid, 2.2; pyridoxine HCl, 11; thiamin mononitrate, 11.

[c] Ascorbate-2-polyphosphate (Stay C®) Hoffmann LaRoche, Inc., Nutley, New Jersey, USA.

[d] Minerals supplied (mg/kg diet): cobalt, 0.05; iodine, 2.4; zinc, 200; selenium, 0.1; manganese, 25; iron, 30; copper, 5.

[e] 98.5% lysine, Archer-Daniels-Midland (ADM), Decatur, Illinois, USA.

[f] Estimated from free gossypol concentration of cottonseed meal used in the study. Cottonseed meal contained about 800 mg/kg free gossypol.

25% cottonseed meal; diet 3, 37.5% cottonseed meal; and diet 4, 52% cottonseed meal. Feeds were manufactured every 2 mo during the experiment in the experimental feed mill located at Delta Western Research Center (DWRC), Indianola, Mississippi, USA. Dietary protein level was determined using an automated Kjeldahl method (Association of Official Analytical Chemists 1990).

The study was conducted in 0.04-ha earthen ponds located at the DWRC and leased by the Mississippi Agricultural and Forestry Experiment Station (MAFES). Male channel catfish (average initial weight = 1.2 kg) were stocked in April 1992 at a rate of 120 fish per pond. Two ponds were used for each treatment. The fish were fed daily to satiation during the growing season, and at the rate of 0.5% body weight during winter months on days when water temperature exceeded 15 C. The experimental period ended July 1994.

Water temperature and dissolved oxygen (DO) were measured daily and throughout the night, except during winter months, using an oxygen/temperature meter (YSI, Yellow Springs, Ohio, USA). Supplemental aeration (provided by an electrical aerator) was used when DO concentrations dropped to 4 mg/L. Ammonia, nitrite, and pH were measured biweekly using a Hach DR-EL/2 spectrophotometer (Hach Chemical Co., Ames, Iowa, USA). All water quality characteristics were in a range suitable for growth of channel catfish (Tucker and Robinson 1991).

At the end of the study, all fish were harvested and total number and weight of fish in each pond were determined. Fourteen healthy, mature male channel catfish from each pond (112 total) were killed and tissue collected to evaluate anterior and total testis weight. Initial and 24 h sperm motilities were also measured (Sneed and Clemens 1963). Testes were removed and placed into Hanks' balanced salt solution (HBSS) (Tiersch et al. 1994) and stored chilled until processed. The osmotic pressure of all solutions was adjusted to >280 mOsm to en-

TABLE 2. *Mean initial weight, final weight, weight gain, feed consumption, feed conversion ratio (FCR), and percent survival of channel catfish from two replicate ponds fed diets containing different percentages of cottonsyed meal (% CSM). Means within a column sharing a common letter were not significantly different.*[1]

Treatment (% CSM)	Initial wt. (kg/fish)	Final wt. (kg/fish)	Weight gain (kg/fish)	Feed consumption (kg/pond)	FCR[2]	Survival (%)
0	1.18	2.45a	1.27a	790	4.2	96a
25	1.27	2.36a	1.09ab	786	4.2	88ab
37.5	1.27	2.09b	0.82b	871	5.9	86b
52	1.23	2.09b	0.91b	774	6.1	92ab

[1] Pooled standard errors for initial weight, final weight, weight gain, feed consumption, FCR, and survival were 0.06, 0.06, 0.08, 40, 0.6, and 2, respectively.

[2] FCR = feed fed/weight gain.

TABLE 3. *Mean concentration of free gossypol in liver tissue of channel catfish fed diets containing either 0 or 50% cottonseed meal (CSM). Means within a column sharing a letter were not significantly different.*[1]

	Gossypol concentration (microgram/gram dry tissue)		
Treatment	(+) isomer	(−) isomer	Total
No CSM	0.32b	0.00b	0.32b
52% CSM	32.40a	21.83a	54.23a

[1] Pooled standard errors for + isomer, − isomer, and total gossypol concentrations were 2.72, 2.18, and 4.89, respectively.

sure that sperm did not activate and become motile during storage (Bates et al., in press). Livers were removed, placed into plastic bags and frozen at −80 C for subsequent gossypol analysis.

Processing of tissue consisted of weighing the anterior portion and total testis, dissociating the testis to release sperm into HBSS (1 g of testis/20 mL HBSS) and estimating motility. Posterior testis weight, the ratio of mass of anterior and posterior testis, and gonosomatic index (GSI) were calculated from the measurements. Anterior and posterior testes were differentiated visually by coloration. The anterior portion was filled with sperm and was white; whereas, the posterior portion was reddish pink. The percentage of actively swimming sperm was estimated for each sample by placing 1 μL of sperm and 20 μL of water on a microslide and viewing under dark-field microscopy at 100× magnification. Motile sperm (actively swimming in a forward direction) were counted by visual detection (Bates et al., in press). All testes were placed into plastic storage bags and refrigerated, and processed within 1 h of removal from the fish (Christensen and Tiersch, in press). Motility estimates made at the time of processing were referred to as the initial motility. Motility was also estimated after 24 h of storage at 4 C.

Liver tissue was freeze dried and gossypol concentrations were determined by high pressure liquid chromatography (HPLC) at the Texas Agricultural Experiment Station, San Angelo, Texas, USA (Kim and Calhoun 1995). Gossypol was determined in liver tissue of fish fed the basal diet (no cottonseed meal) and those fed the diet containing the highest level (52%) of cottonseed meal.

All data were analyzed using the Statistical Analysis System (SAS/STAT edition 6.04). A one-way analysis of variance and Duncan's new multiple range test were used to determine statistical differences. Data expressed as percentages were arcsine transformed before analysis. Differences were considered significant at $P < 0.05$.

Results and Discussion

Feed Consumption. Feed Conversion, and Weight Gain

Fish fed diets containing either 37.5% or 52% cottonseed meal consumed as much feed as fish fed either the basal diet (no cottonseed meal) or a diet containing 25% cottonseed meal, but appeared to convert feed less efficiently and thus gained less weight (Table 2). Feed conversions were high in all treatments but tended to be highest in fish fed diets containing the two highest levels of cottonseed meal (Table 2). A significant difference in feed conversion was not evident at $P < 0.05$, but feed conversion of

TABLE 4. *Means of selected reproductive parameters from channel catfish fed diets containing different percentages of cottonseed meal (% CSM). Means within a column sharing a letter were not significantly different.*[1]

Treatment % CSM	Fish wt. kg	Testis wt. (g) Anterior	Posterior	Total	GSI[2]	Ant./Post. ratio	Sperm motility (%) Initial	24 h
0	2.6a	7.13	4.44	11.57	0.47	2.04	59b	61
25	2.8a	6.92	5.28	11.98	0.47	1.99	53b	51
37.5	2.2b	7.60	5.01	12.61	0.61	1.98	65ab	67
52	2.2b	6.74	5.57	12.31	0.60	1.44	73a	71

[1] Pooled standard errors for fish weight, anterior testis weight, posterior testis weight, total testis weight, GSI, anterior/posterior ratio, and sperm motility initial and 24 h were, 0.11, 0.66, 0.51, 0.59, 0.04, 0.22, 4.4, and 6.1, respectively.

[2] GSI (Gonosomatic Index) = total testis weight/fish weight.

fish fed diets containing 37.5% and 52% cottonseed meal were significantly higher at $P < 0.10$. Large fish generally convert feed less efficiently than do small fish, and are more difficult to feed because they are wary and do not feed as readily. Thus a feed conversion of 3 to 5 is not uncommon for catfish broodstock (Robinson et al. 1994).

The higher feed conversions of fish fed high levels of cottonseed meal were unexpected and the response is difficult to explain based on current literature on the use of cottonseed meal in the diets of food-sized fish. Levels of cottonseed meal up to 40% of the diet have not been shown to be detrimental to growth of channel catfish foodfish; however, levels of 50% cottonseed meal depressed weight gain unless the diet was supplemented with lysine (Li and Robinson 1993; Robinson and Li 1993). In the present study, all diets containing cottonseed meal were supplemented with lysine at a level necessary to meet the dietary requirement for channel catfish (National Research Council 1993). The diets were adequate in all nutrients known to be essential for the grow out of channel catfish (National Research Council 1993). Estimated digestible energy levels of diets containing cottonseed meal (2.7 kcal/kg diet) were slightly less than that of the basal diet (2.8 kcal/kg). However, it is unlikely that this contributed to the poor feed conversion and weight gain of fish fed diets containing cottonseed meal because growth of fish fed a diet containing 25% cottonseed meal was not different than that of fish fed the basal diet.

Another factor that may have affected growth is the level of free gossypol in the diet. However, unless broodfish are more sensitive to free gossypol than are fingerling or foodfish, it is unlikely that free gossypol reduced growth rate. A level of 900 mg/kg free gossypol depressed growth in fingerling channel catfish (Dorsa et al. 1982). Levels of free gossypol up to 500 mg/kg have not affected growth of food-sized channel catfish (Li and Robinson 1993; Robinson and Li 1993). The concentration of free gossypol in diets used in the present study did not exceed 400 mg/kg. The free gossypol concentration in the liver of fish fed the diet containing 52% cottonseed meal (Table 3) was considered to be relatively low compared to that seen in other animals (Millard Calhoun, personal communication).

There does not appear to be a clear explanation for the lower growth and feed conversion observed in fish fed diets containing high levels of cottonseed meal in the present study. It may be related to nutrition, since the nutrient and energy requirements of channel catfish broodfish are not known with any precision, nor is it known if broodfish are more sensitive to the toxic effects of free gossypol.

Testis Weight. GSI. and Sperm Motility

Channel catfish in this study were sampled during spawning season and therefore possessed well-developed testes. Sperm motility values were all above 50% (Table 4) indicating that, in general, sperm samples from all treatments were of good quality. Sperm motility was significantly higher in fish receiving diets containing the highest levels of cottonseed meal (37.5% and 52%). Total weights and the weights of the anterior and posterior portions of the testis were not different among treatments. The mean values for total testis weights were all around 12 g (Table 4) which indicated good testis and sperm development in all treatments. On average, the anterior testis was double the size of the posterior testis. There were no differences in testes in relation to their body size (GSI values) regardless of diet. Long-term feeding of cottonseed meal diets appeared to have no negative effect on testis weight and sperm motility in channel catfish broodfish.

Summary

Testis weight and sperm motility were not adversely affected by high levels of cottonseed in the diet indicating that reproductive capacity was not diminished. Although feed conversion increased and weight gain decreased in fish fed high levels of cottonseed meal, growth of broodfish is not critical and a slower growth rate has little practical significance. At any rate, it is unlikely that levels of cottonseed meal in catfish feeds will exceed 25% to 30% because of economic considerations; thus, adverse effects on growth or on reproductive capacity of males due to the inclusion of cottonseed meal is unlikely.

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