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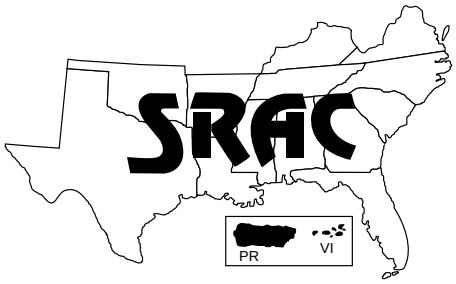

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Aquaculture Food Safety—Residues

Final Project Report on the
SRAC Regional Research Project

"Aquaculture Food Safety—Residues"

SRAC No. 6001

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Preface

The project summarized in this report was developed and funded through the Southern Regional Aquaculture Center, one of five regional aquaculture research and Extension centers established by Congress in 1985 and administered by the United States Department of Agriculture. The five centers are located in the northeastern, north-central, southern, western and tropical Pacific regions of the country. The Southern Regional Aquaculture Center began organizational activities in 1987, and the first research and Extension projects were initiated in 1988. The thirteen states and two territories included in the Southern Region are Alabama, Arkansas, Florida, Georgia, Kentucky, Louisiana, Mississippi, North Carolina, Oklahoma, Puerto Rico, South Carolina, Tennessee, Texas, U.S. Virgin Islands and Virginia.

The regional aquaculture centers encourage cooperative and collaborative research and Extension educational programs that have regional or national applications. Center programs complement and strengthen research and Extension educational programs provided by the Department of Agriculture and other public institutions.

The mission of the centers is to support aquaculture research, development, demonstration and Extension education, and to enhance viable and profitable domestic aquaculture production for the benefit of consumers, producers, service industries, and the American economy. Projects developed and funded by the centers are based on regional industry needs and are designed to aid commercial aquaculture in all states and territories. The centers are organized to take advantage of the best aquaculture science, education skills, and facilities in the United States. Center programs ensure effective coordination and a region-wide, team approach to projects jointly conducted by research, Extension, government and industry personnel. Interagency collaboration and shared funding are strongly encouraged.

Project Participants

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Contents

List of Tables

List of Figures

Executive Summary

The research described in this report represents the efforts of scientists from eight universities. The primary objectives were to develop analytical methods for measuring antibiotic residues in catfish and use those methods to determine residues in fish exposed under controlled conditions; to survey catfish, trout and crayfish from production and processing facilities in order to determine concentrations of selected organochlorines, organophosphates, pyrethroids and heavy metals; to determine the effects of baking, frying and smoking on pesticide and antibiotic residues; and to determine the influence of antibiotics on catfish growth rate. It is hoped that this work will help the aquaculture industry maintain the safety of products sold in grocery stores and restaurants.

Method Development for Antibiotic Residue Testing

A useful liquid chromatography method for measuring oxytetracycline, sulfadimethoxine and ormetoprim in catfish fillets was developed. Mean recovery of oxytetracycline in the range of 0.05 to 1.0 ppm was 92.5 percent. In the range of 0.05 to 5.0 ppm, recovery was 86.3 percent for ormetoprim and 87.9 percent for sulfadimethoxine. The method described in this report is useful for determining residues of these antibiotics at levels lower than the established tolerances.

Capillary electrophoresis was found to be an acceptable method of measuring oxytetracycline in fish tissue. Capillary electrophoresis requires less sample volume and less solvent than liquid chromatography. The method described here had an elution time for oxytetracycline of 10.9 minutes, which is comparable to liquid chromatography, and a recovery of 92.9 percent in the range from 0.1 to 25 ppm.

Antibiotic Residues in Channel Catfish

Residues of oxytetracycline were detected immediately after 4 or 8 weeks of feeding fingerling channel catfish the drug at 12.5, 25 and 50 mg/kg body weight, but residues were not detected after a 3-week withdrawal period. Sulfadimethoxine and ormetoprim were rapidly depleted from food-sized channel catfish after 5 days of feeding Romet-30 (the commercial 5:1 mixture of the two drugs) at 50 to 100 mg/kg body weight. No residues were detected 2 days post-treatment.

Pesticide and Heavy Metal Residues in Catfish, Trout and Crayfish

Channel catfish (n = 257), rainbow trout (n = 33) and red swamp crayfish (n = 38) were collected from production and processing (catfish only) facilities located in eight southern states over a 3-year period. Approximately 45 percent of catfish, 73 percent of trout and 92 percent of crayfish contained no detectable residues of 34 organochlorines, organophosphates or pyrethroids. With one exception (chlorpyrifos), all residues were well within recommended limits. Furthermore, residues of nine heavy metals were below federal action limits. This research is the most comprehensive survey of aquaculture products and demonstrates the low levels of contaminants in these three species.

Influence of Cooking Processes on Pesticide and Antibiotic Residues

Channel catfish were fed diets fortified with p,p'-DDE, dieldrin, chlordane, toxaphene, ormetoprim/sulfadimethoxine, or oxytetracycline. From each fish, one raw fillet and one baked, smoked or fried fillet were analyzed for residues and compared.

In one study, smoking caused the greatest reduction in DDE residues (82 percent), while baking caused the least reduction in DDE (50 percent) and dieldrin (49 percent) residues when data were reported on a dry weight basis. In a second study, smoking reduced chlordane by 9 percent and toxaphene by 24 percent, while frying reduced chlordane by 56 to 86 percent and toxaphene by 40 to 49 percent. In a third study, cooking caused a 54 percent reduction in ormetoprim and a 46 percent reduction in sulfadimethoxine; however, the effects from the

cooking methods (baking, smoking or frying) were not significantly different. In a fourth study, frying reduced oxytetracycline by 44 to 49 percent, smoking by 72 percent and baking by 77 percent.

Overall, cooking reduced pesticide and antibiotic residues in catfish fillets between 9 and 86 percent depending on the chemical and the cooking technique. These data can be helpful when attempting to perform risk assessment related to human exposure to contaminants in fish.

Influence of Antibiotics on Fish Growth

Adding antibiotics to the diets of developing catfish did not improve growth rate when feed that was fortified with either OTC or a SDM and OMP mixture was compared to a control diet.

Recommendations

Antibiotics

- Producers need to observe dosage levels and strictly adhere to withdrawal times for the approved antibiotics.
- Antibiotics should not be used to increase fish growth rate since data presented here indicate that these antibiotics may slow fish growth and overuse of antibiotics may promote the development of resistant pathogens.
- Rapid assays should be established as part of the HACCP program to discourage improper use of antibiotics on the farm.
- Funds should be secured to survey aquaculture products for antibiotic residues.

Pesticides

- Educational programs should be developed regarding the importance of using only approved pesticides in and around ponds.
- Funding should be provided for the development of rapid assays that could be used by processors to screen for pesticides at the point of purchase.
- Producers should keep records on feeds (lot numbers and manufacturer names) since the primary route of exposure to pesticides is the fish's diet.
- Additional funding should be found to test for pesticides that are not detected using a multiresidue method.

Metals

- The aquaculture industry should continue to survey fish and feeds for mercury, chromium, arsenic and lead.

Project Background

Seafood safety is a concern to consumers and to the companies that produce, process and prepare seafood products. The growth of the aquaculture industry has stimulated the implementation of fish and seafood products inspection programs by the U.S. Department of Agriculture (USDA), the National Oceanic and Atmospheric Administration (NOAA), and the Food and Drug Administration (FDA). The mandatory inspection incorporates a Hazard Analysis Critical Control Point (HACCP) strategy for control of human pathogens and chemical contaminants. There is always a potential for seafood to be contaminated by pesticides, heavy metals and pharmaceutical compounds either from direct or indirect sources. These potential problems can occur on the farm, during processing, or at the wholesale/retail levels. One way to ensure safe food for the consumer is to generate data on the chemicals that may enter aquaculture products during production and processing.

This project is the result of the SRAC Industry Advisory Committee's concern about consumers' perceptions of seafood safety. The Industry Advisory Committee asked the SRAC Board of Directors to place a high priority on food safety and initiate a regional project. The purpose of this project is to identify real or potential problems and help the industry continue to ensure that safe, high quality products are in the market place.

Project Objectives

Objective 1. Develop a method of testing for antibiotic residues

Objective 2. Determine the antibiotic residues in channel catfish

Objective 3. Measure metal and pesticide residues in aquaculture products (channel catfish, rainbow trout and red swamp crayfish)
- a. Heavy metals
- b. Organochlorines, organophosphates and pyrethroids

Objective 4. Determine the influence of cooking processes on pesticide and antibiotic residues
- a. Dieldrin, p,p'-DDE, chlordane and toxaphene
- b. Ormetoprim and sulfadimethoxine
- c. Oxytetracycline

Objective 5. Determine the effects of antibiotics on fish growth

Objective 1: Develop a method of testing for antibiotic residues

Currently, two antibiotic drugs are approved for use in channel catfish production—Terramycin (oxytetracycline) and Romet-30 (sulfadimethoxine and ormetoprim in a 5:1 ratio). By law, the withdrawal time for oxytetracycline (OTC) is 21 days and the withdrawal time for sulfadimethoxine (SDM) and ormetoprim (OMP) is 3 days.

There are no official methods for determining OTC, SDM or OMP residues in catfish. Most classic assays are time consuming and lack specificity. Liquid chromatography, however, is a selective and sensitive method commonly used to measure antibiotic residues in a variety of animal products. One goal of this study was to develop liquid chromatography procedures for measuring OTC, SDM or OMP residues in catfish tissue.

Capillary electrophoresis has many benefits over liquid chromatography, such as shortened separation time and reduced solvent use. However, capillary electrophoresis is not a standard method for determining residues in fish. Therefore, another goal of this study was to use capillary electrophoresis in determining oxytetracycline residues in raw and cooked channel catfish.

Using Liquid Chromatography to Measure Antibiotics

Analytical Methods

Channel catfish fillets were purchased from a retail outlet and fortified with antibiotics to obtain OTC, OMP or SDM levels of 0 to 5.0 ppm. Channel catfish treated with antibiotics were also obtained from Texas A&M and Auburn Universities. Fillets were shipped on dry ice to the University of Florida for analysis.

Oxytetracycline was extracted from 5 g of homogenized fish tissue using 2 mL of 50% trichloroacetic acid, 30 ml of 1 M HCl, and 0.5 g EDTA. The mixture was shaken and centrifuged, and the supernatant was passed through a C18 solid phase extraction column and eluted with methanol.

Extracts were analyzed using liquid chromatography with an isocratic acetonitrile (17 parts)/ 0.02 M phosphate buffer (83 parts) mobile phase. Analytes were separated and quantified using an ODS column (5 µm particle size) with a tunable absorbance detector set at 353 nm.

Ormetoprim and sulfadimethoxine were extracted from 5 g homogenized fish tissue with 2 ml of 0.05 M potassium carbonate/potassium borate/potassium hydroxide buffer, 1 ml of 1 N sodium hydroxide, 400 µl of 1 M tetrabutylammonium hydroxide in methanol, and 25 ml methylene chloride. The mixture was shaken, centrifuged, filtered and analyzed using the same column as for OTC but with a mobile phase of a 20:2:76:2 v/v mixture of acetonitrile/methanol/0.1 M phosphate buffer/1-heptanesulfonic acid. The detector was set at 272 nm.

Additional information on these methods can be found in:

W. X. Du, M. R. Marshall, W. B. Wheeler, M. Mathews, D. Gatlin, S. D. Rawles, D. -H. Xu, W. A. Rodgers and C. I. Wei, 1995, Oxytetracycline, Sulfadimethoxine, and Ormetorprim Residues in Channel Catfish by HPLC, *Journal of Food Science* 60:1220-1224 & 1227.

Results

Retention time for OTC was 6.9 minutes with a lower limit of detection of 0.05 ppm for catfish extracts. Variations in intra-assay (average coefficient of variation was 4.7 percent) and inter-assay (average coefficient of variation was 6.3 percent) were utilized to verify the reproducibility of the method. The mean recovery for OTC in the 0.05 to 1.0 ppm range was 92.5 percent. Retention times for SDM and OMP were 14.5 and 7.2 minutes, respectively, with a detection limit of 0.05 ppm for both SDM and OMP. The mean recovery in the 0.05 to 5.0 ppm range was 87.9 percent for SDM and 86.3 percent for OMP. The method described in this report is useful for determining residues of OTC at levels lower than the established tolerance of 0.1 ppm.

Using Capillary Electrophoresis to Measure Oxytetracycline

Analytical Methods

Channel catfish were fed for 10 days on diets fortified with OTC to provide dosages of 37.5, 75.0 and 150.0 mg/kg of body weight. Fish were sacrificed 18 hours after the last feeding and two fillets collected for analysis. One fillet was kept raw and one was cooked. Four cooking methods were used: breaded then deep fat fried; injected with 6 percent phosphate solution then fried; baked; and smoked. To compare extraction results, catfish fillets were purchased; some were injected with OTC at 0.05, 0.1, 0.2, 0.5, 1.0, 2.0 and 5.0 ppm, and the rest were used as blanks.

Oxytetracycline was extracted from the samples as described above for the liquid chromatography method and then concentrations were determined using capillary electrophoresis with a 25 cm × 25 µm capillary column under 8 kV constant voltage.

Additional information on this study is available in:

Huang, T.S., W.X. Du, M. R. Marshall, and C.I. Wei, 1997, Determination of Oxytetracycline in Raw and Cooked Channel Catfish by Capillary Electrophoresis, *Journal of Agricultural and Food Chemistry* 45:2602-2605.

Results

The limit of detection for OTC in catfish was 0.1 ppm. The electrophoretograms from the study showed a migration time of 10.9 minutes and a linear range from 0.1 to 25 ppm for OTC standard solutions. The mean recovery rate was 92.9 percent for the 0.1 to 25.0 ppm range. The comparison of liquid chromatography and capillary electrophoresis in Table 1 demonstrates good correlation between the two methods.

Table 1. Oxytetracycline (OTC) residues in catfish as determined by capillary electrophoresis (CE) and liquid chromatography (LC).

Feed fortification level (ppm)	CE	LC
	OTC* (ppm)	OTC** (ppm)
0.1	0.094	0.099
0.5	0.468	0.443
1.0	0.939	0.861

*Data from Du et al. (1997)
**Data from Du et al. (1995)

Conclusions

The liquid chromatography methods developed in these studies provided excellent recoveries and reproducible results at residue levels well below tolerance levels. The single extraction, single wavelength method developed for SDM and OMP is more convenient than separate procedures for the two drugs. Capillary electrophoresis was shown to be an effective method for determining OTC residues in catfish tissue. The method has certain operational advantages over liquid chromatography and can be used as an alternative procedure for determination of residues.

Objective 2: Determine the Antibiotic Residues in Channel Catfish

Treating fish with antibiotics may cause residues in the meat unless an appropriate withdrawal time is observed. Antibiotic residues in foods are a potential health threat, especially to people who are allergic to certain antibiotics. The FDA has established withdrawal times of 21 days for oxytetracycline (OTC) and 3 days for Romet-30 (sulfadimethoxine [SDM] and ormetoprim [OMP]).

Although pharmacokinetic studies of OTC, SDM and OMP have been conducted for channel catfish under laboratory conditions, there is no information on residues in fish under commercial feeding conditions. The following two studies were conducted to determine residues in fish exposed to the drugs under controlled, practical conditions.

Additional information on these studies is available in:

W. X. Du, M. R. Marshall, W. B. Wheeler, M. Mathews, D. Gatlin, S. D. Rawles, D. -H. Xu, W. A. Rodgers, and C. I. Wei, 1995, Oxytetracycline, Sulfadimethoxine, and Ormetorprim Residues in Channel Catfish by HPLC, *Journal of Food Science* 60:1220-1224 & 1227.

Oxytetracycline, Sulfadimethoxine and Ormetorprim Residues in Fingerling Channel Catfish

Methods

Juvenile channel catfish (20.2 to 20.4 g initial body weight) were conditioned for 2 weeks, then randomly assigned to groups and fed one of six treatment diets containing SDM/OMP or OTC. Treatment diets were prepared by adding SDM/OMP or OTC to the base diet to provide dosages of 12.5, 25 or 50 mg/kg body when fed at 3 percent body weight per day. Catfish remained on the treatment diets for 8 weeks, followed by a 3-week withdrawal period. After 4 and 8 weeks of feeding and then again after 3 weeks of withdrawal, two fish from each group were sacrificed, skinned and filleted. Fillet samples were wrapped separately and tissue was frozen for analysis by the liquid chromatography method described in Objective 1.

Results

The control groups had no detectable OTC residues, whereas catfish fed OTC for 4 weeks at 12.5, 25.0 and 50.0 mg/kg had average OTC residues of 0.06, 0.12 and 0.16 ppm, respectively. Catfish fed for 8 weeks averaged 0.06, 0.10 and 0.22 ppm, respectively (Table 2). There were no significant differences ($p<0.05$) in residues from catfish fed for 4 weeks and catfish fed for 8 weeks; however, the residues from the three dosage levels were significantly different. Treated fish had no detectable OTC residue after the 3-week withdrawal period.

SDM and OMP residues in catfish fed Romet-30 are shown in Table 2. The average residue levels for catfish fed SDM for 4 weeks at 12.5, 25.0 and 50.0 mg/kg were 0.20, 0.37 and 0.74 ppm, respectively. In comparison, catfish fed for 8 weeks had average residue levels of 0.31, 0.62 and 2.16 ppm, respectively. OMP residue levels for the corresponding dosages were 0.03, 0.05 and 0.17 ppm. No OMP or SDM was detected in any fish after the 3-week withdrawal period.

Sulfadimethoxine and Ormetorprim Residues in Food-sized Channel Catfish

Methods

Romet-30 is the most commonly used antibiotic to treat ESC (enteric septicemia of catfish); its mandatory withdrawal period is 3 days. This study was conducted to determine residues in food-sized catfish 1 to 5 days after a 5-day feeding regime on medicated feed (which is the labeled treatment protocol).

Channel catfish (345.0 ± 11.6 g) were divided into four groups and conditioned for 15 days. Four diets were formulated to deliver SDM/OMP at 0, 50, 75 and 100 mg/kg body weight when fed at 2 percent body weight per day. Fish were fed the experimental diets for 5 days. At 24, 48, 72, 96 and 120 hours after their last feeding, three fish from each group were sacrificed, skinned and filleted. Tissue was frozen for analysis.

Table 2. Average concentrations of oxytetracycline (OTC), ormetoprim (OMP) and sulfadimethoxine (SDM) in channel catfish after 4 and 8 weeks of feeding OTC or OMP and SDM.

Feeding period	Dose (mg/kg)	OTC (ppm) Mean ± SD	OMP (ppm) Mean ± SD	SDM (ppm) Mean ± SD
4 weeks	Control	ND*	ND*	ND*
	12.5	0.06 ± 0.02^{a}	0.01 ± 0.02^{a}	0.20 ± 0.18^{a}
	25.0	0.12 ± 0.04^{bc}	0.04 ± 0.05^{a}	0.37 ± 0.39^{a}
	50.0	0.16 ± 0.04^{c}	0.14 ± 0.21^{a}	0.74 ± 1.11^{a}
8 weeks	Control	ND*	ND*	ND*
	12.5	0.06 ± 0.02^{a}	0.03 ± 0.02^{a}	0.31 ± 0.33^{a}
	25.0	0.10 ± 0.03^{b}	0.05 ± 0.03^{bc}	0.62 ± 0.27^{ab}
	50.0	0.22 ± 0.15^{c}	0.17 ± 0.14^{c}	2.16 ± 1.60^{c}

*ND = not detected

$^{a\text{-}c}$ = Means with different letters are significantly different ($p<0.05$).

Results

Of 45 fish analyzed at 24 hours after their last feeding, four fish contained OMP or SDM residues that exceeded the 0.1 ppm tolerance limits. No residue was detected in any fish 48 hours, or longer, after their last feeding with medicated feed.

Conclusions

Residues of oxytetracycline, sulfadimethoxine and ormetoprim were detected in catfish immediately after fish completed medicated feed therapy. However, residues were not present after the prescribed withdrawal periods (21 days for OTC and 3 days for SDM and OMP). These studies verify the effectiveness of the mandatory withdrawal periods in eliminating problems with antibiotic residues in farm-raised channel catfish and emphasize the need for producers to adhere to those withdrawal periods before fish are offered for slaughter.

Objective 3: Measure Metal and Pesticide Residues in Aquaculture Products

While numerous studies have demonstrated the contamination of sportfish with heavy metal and pesticide residues, there has been little research on the level of contaminants in farm-raised fish. Higher quality groundwater is usually used in production environments and fish are fed a commercial feed, so farm-raised fish would be expected to have much lower residue levels than sportfish. Conversely, farmed fish tend to have higher fat content than their wild counterparts, and this means they have a larger reservoir in which to accumulate lipophilic contaminants. The purpose of the following research was to determine the levels of selected metals and pesticides in farm-raised channel catfish, rainbow trout and red swamp crayfish from southern aquaculture operations.

Methods

Fish tissue was collected quarterly during a 3-year period from production and processing facilities in Georgia, Florida, Tennessee, North Carolina, Alabama, Mississippi, Louisiana and Texas to provide a total of 328 samples (Table 3). Channel catfish were collected from production facilities (196 samples) and from processing facilities (61 samples). Rainbow trout (33 samples) and red swamp crayfish (38 samples) were collected only from production facilities. Also collected were catfish and trout fillets that included belly-flaps (10 samples 0.5 to 1 kg in size; approximate length of 35 to 41 cm), and 5 kg samples of raw, unpurged crayfish tail meat (including vein). These samples were harvested from production sites. In addition, 5-kg frozen catfish fillets were collected from processing sites. Samples were frozen, encoded and sent to a central processing facility where the fish samples were re-encoded and prepared for analysis. Thirty-four organochlorine, organophosphate and pyrethroid compounds and nine heavy metals were measured (Table 4).

Additional information on these studies can be found in:

Santerre, C.R., P.B. Bush, D. Xu, G.W. Lewis, J.T. Davis, R.M. Grodner, R. Ingram, C.I. Wei and J. Hinshaw. 2001. Metal Residues in Farm-raised Channel Catfish, Rainbow Trout, and Red Swamp Crayfish From the Southern U.S. *Journal of Food Science* 66(2):270-273.

Santerre, C.R., R. Ingram, G.W. Lewis, J.T. Davis, L.G. Lane, R.M. Grodner, C.I. Wei, P.B. Bush, D. Xu, J. Shelton, E.G. Alley and J.M. Hinshaw. 2000. Organochlorines, Organophosphates and Pyrethroids in Channel Catfish, Rainbow Trout, and Red Swamp Crayfish from Aquaculture Facilities. *Journal of Food Science* 65(2):231-235.

Table 3. Origin of samples used to assess chemical residues in channel catfish, rainbow trout and red swamp crayfish.

	Channel catfish			Rainbow trout (33)	Red swamp crayfish (38)
	Producer (197)	Processor (60)	Total (257)		
Alabama	23	17	40	0	0
Florida	39	4	43	0	0
Georgia	31	0	31	15	0
Louisiana	28	13	41	0	23
Mississippi	33	23	56	0	0
North Carolina	0	0	0	13	0
Tennessee	3	0	3	5	0
Texas	40	3	43	0	15

Table 4. Possible residues of environmental contaminants measured in channel catfish, rainbow trout and red swamp crayfish.

Organochlorines
Polychlorinated biphenyls (PCB) - Aroclor 1242*, 1248*, 1254*, 1260*
Hexchlorobenzene$^{\varphi}$
Mirex$^{\varphi}$
Heptachlor$^{\varphi}$ and heptachlor epoxide$^{\varphi}$
Methoxychlor$^{\varphi}$
Dieldrin$^{\varphi}$ and endrin$^{\varphi}$
Endosulfan 1$^{\varphi}$, 11$^{\varphi}$ and sulfate$^{\varphi}$
Dichlorodiphenyltricholoroethane (DDT) - o,p'-DDD$^{\varphi}$, o,p'-DDE$^{\varphi}$, p,p'-DDD$^{\varphi}$, p,p'-DDE$^{\varphi}$, p,p'-DDT$^{\varphi}$ and p,p'-DDD olefin$^{\varphi}$)
Benzene hexachloride (α-, β-, γ-BHC {lindane})$^{\varphi}$
Chlordane (oxychlordane$^{\varphi}$, trans-nonachlor$^{\varphi}$, α- and γ-chlordane$^{\varphi}$)
Organophosphates
Chlorpyrifos-ethyl**
Diazinon**
Malathion**
Methyl-parathion**
Ethyl-parathion**
Pyrethroids
Cypermethrin*
Fenvalerate*
Heavy Metals
Barium§
Cadmium§
Copper§
Chromium§
Silver§
Lead*
Arsenic$^{\in}$
Selenium$^{\omega}$
Mercury$^{\sigma}$
Lower limit of detection (ppm) = $^{\sigma}$0.001; $^{\varphi}$0.01; **0.02; $^{\omega}$0.033; $^{\in}$0.04; *0.05; §0.1

Results

Heavy Metals in Farm Raised Channel Catfish, Rainbow Trout and Red Swamp Crayfish

Results of metal residue assays are provided in Table 5. Barium (Ba) residues in catfish from production facilities were detected in 12 percent of the samples and averaged 0.27 ppm. Of the catfish from processing facilities, 5 percent contained Ba residues, with an average of 0.12 ppm. Ba residues were found in 15 percent of the rainbow trout samples, with an average concentration of 0.16 ppm, and 95 percent of crayfish, at an average of 1.25 ppm. For all of the metals assayed in this research, except for mercury, there are no national action limits to restrict the amount that can be found in fish. It is important to note that some of the compounds tested in this study are essential nutrients and their inclusion in the list was a matter of convenience relative to the chosen test method. However, the Environmental Protection Agency's Region III office has set values that can be used as a guide when interpreting metal concentrations in fish. These advisory limits are referred to as the Risk Based Concentration (EPA 1999). For Ba, the limit has been set at 95 ppm. The highest concentration of Ba detected in any sample was less than 5 percent of the limit.

Cadmium (Cd) residues in catfish from production sites were detected in 2 percent of the samples, with an average concentration of 0.48 ppm. Cd was not detected in catfish samples from processing facilities, in rainbow trout samples or in red swamp crayfish samples. The Risk Based Concentration limit is 1.4 ppm, while the World Health Organization (WHO) has set a limit of 8.0 ppm (Okoye, 1994). There were only three catfish samples that had detectable residues, with the highest being measured at 45 percent of the limit. The United Nations/Food and Agriculture Organization (FAO) has set a limit for Cd of 0.5 ppm (Abou-Arab et al., 1996).

Copper (Cu) was measured in 83 percent of catfish samples from pond sites, with an average concentration of 0.27 ppm, compared to 85 percent in catfish from production facilities, with an average concentration of 0.23 ppm. Eighty-eight percent of rainbow trout samples had an average Cu concentration of 0.36 ppm. Cu plays an important role in oxygen transportation in crayfish, much as iron functions in hemoglobin in humans; thus it was found in 100 percent of the crayfish. The average concentration in crayfish was 4.96 ppm. The Risk Based Concentration limit is 54 ppm. The highest crayfish sample was 25 percent of the limit. The WHO limit is 120 ppm and the 1983 FAO limit is 20.0 ppm.

Chromium (Cr) was detected in 20 to 26 percent of the catfish samples, with an average concentration of 0.31 ppm. Average residues in trout and crayfish were 0.36 and 0.27 ppm, respectively. The Risk

Table 5. Metal residues in channel catfish, rainbow trout and red swamp crayfish.

Metals (LOD)*	Channel catfish		Rainbow trout	Red swamp crayfish
	Producer (n=196)	Processor (n=61)	(n=33)	(n=38)
Barium (0.10 ppm)				
Mean (ppm)	0.268	0.117	0.162	1.246†
Maximum (ppm)	1.34	0.13	0.31	4.61
Number positive (%)	24 (12%)	3 (5%)	5 (15%)	36 (95%)
Cadmium (0.10 ppm)				
Mean (ppm)	0.483	ND*	ND*	ND*
Maximum	0.63	-	-	-
Number positive (%)	3 (2%)	-	-	-
Copper (0.10 ppm)				
Mean (ppm)	0.270†	0.230†	0.360†	4.96†
Maximum (ppm)	0.63	0.48	0.92	13.80
Number positive (%)	162 (83%)	52 (85%)	29 (88%)	38 (100%)
Chromium (0.10 ppm)				
Mean (ppm)	0.316	0.313	0.313	0.273
Maximum (ppm)	1.72	0.84	0.59	0.60
Number positive (%)	51 (26%)	12 (20%)	12 (36%)	16 (42%)
Silver (0.10 ppm)				
Mean (ppm)	0.232	ND*	ND*	ND*
Maximum (ppm)	0.53	-	-	-
Number positive (%)	5 (3%)	-	-	-
Lead (0.50 ppm)				
Mean (ppm)	0.714	0.917	0.724	ND*
Maximum (ppm)	1.00	1.8	1.15	-
Number positive (%)	22 (11%)	7 (12%)	5 (15%)	-
Arsenic (40 ppb)				
Mean (ppb)	75	75	53	80†
Maximum (ppb)	180	150	70	800
Number positive (%)	8 (4%)	6 (10%)	9 (27%)	22 (58%)
Selenium 0.033 ppm)				
Mean (ppm)	0.88†	0.82†	0.163†	0.198†
Maximum (ppm)	0.772	0.153	0.577	0.417
Number positive (%)	158 (81%)	53 (87%)	32 (97%)	37 (97%)
Mercury (1 ppb)				
Mean (ppb)	8.41†	8.37†	9.54†	25.06†
Maximum (ppb)	89	67	34	70
Number positive (%)	165 (84%)	50 (82%)	33 (100%)	37 (97%)
% of Action Limit (1000 ppb)	0.8%	0.8%	0.9%	2.5%

†Means were calculated to include all samples that contained non-detectable (*ND) residues at half of the lower limit of detection. (*LOD). This was done if more than 50 percent of samples had a concentration that exceeded the LOD.

Based Concentration limits are 4.1 for Cr(VI) and 2,000 ppm for Cr(III), which is an essential micronutrient.

Silver (Ag) was detected in only 3 percent of catfish samples from production sites, with an average concentration of 0.23 ppm. The Risk Based Concentration limit is 6.8 ppm.

Lead (Pb) was detected in 11 percent of the catfish from production sites, with an average concentration of 0.71 ppm. Twelve percent of catfish samples from processing facilities had an average concentration of 0.92 ppm. Fifteen percent of trout had an average of 0.72 ppm Pb. A limit of 8.0 ppm has been set by WHO, while the 1983 FAO limit is 2.0 ppm.

Arsenic (As) was detected in 4 percent of catfish samples from production sites, with an average concentration of 75 ppb. Ten percent of catfish from processing facilities had an average arsenic residue of 53 ppb. As was detected in 27 percent of the rainbow trout samples, with an average concentration of 53 ppb, and in 58 percent of crayfish samples, with an average concentration of 80 ppb. The Risk Based Concentration for As is 2.1 ppb, which is below the limit of detection for As in this study. As(III) is considered to be more toxic than As(V) and it is believed that As(V) is the prevalent form in fish. As(V) is believed to be found in a harmless form as arsenobetaine and arsenocholine. Further research on the safety of this metal in foods is necessary.

Selenium (Se) was detected in 81 percent of the catfish samples, with an average concentration of 0.09 ppm. Catfish obtained from processing facilities had detectable levels of Se in 87 percent of the samples, with an average concentration of 0.82 ppm. In both trout and crayfish, Se was detected in 97 percent of the samples, with average concentrations of 0.16 and 0.20 ppm, respectively. The Risk Based Concentration limit is 6.8 ppm.

Mercury (Hg) was detected in 84 percent of the catfish obtained from production facilities, with an average concentration of 8.41 ppb. Samples from processing facilities had Hg residues in 82 percent of catfish, with an average concentration of 8.37 ppb. Trout and crayfish samples were slightly higher, with residues in 100 percent and 97 percent of samples, respectively. Average concentration was 9.54 ppb in trout and 25.6 ppb in crayfish. The Risk Based Concentration for Hg is 140 ppb; however, the FDA Action Limit for mercury in fish is 1,000 ppb. Currently, there is intense debate between FDA (1998) and EPA as to whether the FDA Action Limit is excessively high. In any case, residues in the fish samples tested in this study were 40 to 100 times lower than the FDA Action Limit and 6 to 14 times lower than the EPA's Risk Based Concentration.

Organochlorines, Organophosphates and Pyrethroids in Farm-Raised Channel Catfish, Rainbow Trout and Red Swamp Crayfish

Samples were tested for 34 organic compounds. There were positive responses for DDT, chlordane, PCB, dieldrin, hexachlorobenzene, heptachlor epoxide and chlorpyrifos. All of these compounds, with the exception of chlorpyrifos (commercially sold as Dursban™ or Lorsban™), are no longer in use. For catfish samples, 44.7 percent had no detectable residues, 44.8 percent had detectable residues that did not exceed the action limits, and 10.5 percent had residues of chlorpyrifos that are not permitted in catfish (Figure 1). For trout samples, 73 percent had no detectable residues and 27 percent had detectable residues that were below the action limits. For crayfish, 92 percent had no detectable residues and 7.9 percent had detectable residues that did not exceed the action limit. Contaminant residue data are provided in Table 6.

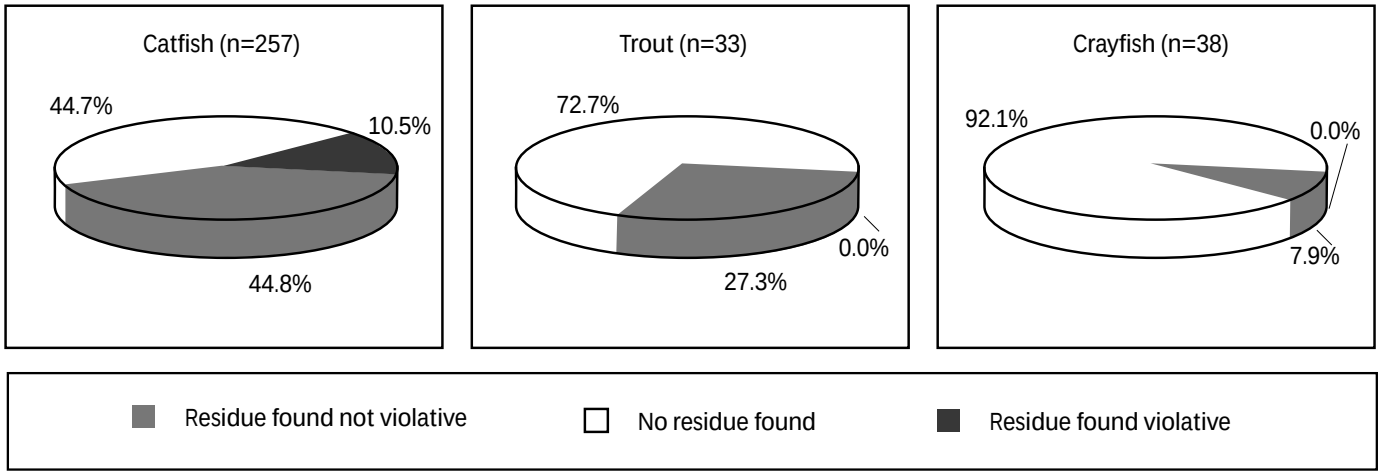

Figure 1. Pesticide residues in channel catfish, rainbow trout and red swamp crayfish.

Table 6. Concentration of chemical contaminants in channel catfish, rainbow trout and red swamp crayfish.

Compound (AL)*	Catfish (n=257)	Trout (n=33)	Crayfish (n=38)
DDT (5 ppm)			
Mean (ppm)	0.043	0.013	0.047
Maximum (ppm)	0.29	0.04	0.11
Number positive (%)	142 (55%)	9 (27%)	3 (8%)
% of Action Limit	0.86%	0.265	0.94%
chlordane (0.3 ppm)			
Mean (ppm)	0.045	0	0
Maximum (ppm)	0.092	0	0
Number positive (%)	5 (2%)	0	0
% of Action Limit	15%	0	0
PCB (2.0 ppm)			
Mean (ppm)	0.133	trace	0
Maximum (ppm)	0.32	trace	0
Number positive (%)	18 (7%)	3 (9%)	0
% of Action Limit	6.65%	trace	0
dieldrin (0.3 ppm)			
Mean (ppm)	0.019	0.01	0
Maximum (ppm)	0.03	0.01	0
Number positive (%)	7 (3%)	1 (3%)	0
% of Action Limit	6.33%	3.33%	0
hexachlorobenzene (none)			
Mean (ppm)	0.01	0	0
Maximum (ppm)	0.01	0	0
Number positive (%)	2 (1%)	0	0
% of Action Limit	NA[d]		
heptachlor epoxide (0.3 ppm)			
Mean (ppm)	0.012	0	0
Maximum (ppm)	0.02	0	0
Number positive (%)	5 (2%)	0	0
% of Action Limit	4.00%		
chlorpyrifos (0 ppm)			
Mean (ppm)	0.072	0	0
Maximum (ppm)	0.37	0	0
Number positive (%)	27 (11%)	0	0
% of Action Limit	>100%	0	0

*AL = FDA Action Limit

DDT was found in 55 percent of catfish, 27 percent of trout and 8 percent of crayfish samples. The most prevalent form of DDT was p,p'-DDE, followed by p,p'-DDD. The FDA Action Limit for the sum of DDT-like compounds is 5 ppm. The residue levels for catfish ranged from 0.01 to 0.29 ppm, with an average of 0.043 ppm, or 0.86 percent of the FDA Action Limit (Table 7). Ranges for DDT-like compounds in rainbow trout and crayfish were 0.01 to 0.04 ppm and 0.01 to 0.11 ppm, respectively. Average concentrations were 0.013 and 0.047 ppm, or 0.26 percent and 0.94 percent of the FDA Action Limit, respectively. Chlordane was detected in 2 percent of catfish samples, with a maximum of 0.092 ppm and an average of 0.045 ppm, or 15 percent of the FDA Action Limit of 0.3 ppm.

Chlordane residues were not detected in trout or crayfish samples.

PCB residues were detected in 7.0 percent of catfish samples and trace levels were detected in 9 percent of trout samples. There were no positive PCB residues detected in crayfish. PCB residues in the catfish ranged from 0.07 to 0.32 ppm, with an average of 0.133 ppm, or 6.65 percent of the FDA Action Limit of 2.0 ppm.

Table 7. FDA Action Limit (AL) for detected pesticides, percent of AL, mean concentration and range for residues in catfish, trout and crayfish.

Contaminant	Action limit (ppm)	Channel catfish			Rainbow trout			Red swamp crayfish		
		Mean* (ppm)	AL# (%)	Rangeϕ (ppm)	Mean* (ppm)	AL# (%)	Rangeϕ (ppm)	Mean* (ppm)	AL# (%)	Rangeϕ (ppm)
DDT	5.0	0.043	0.9	<0.29	0.013	0.3	0.04	0.047	0.9	<0.11
chlordane	0.3	0.045	15.0	<0.09	0	0	0	0	0	0
PCB	2.0	0.133	6.7	<0.32	0	0	0	0	0	0
dieldrin	0.3	0.019	6.3	<0.03	0.01	3.3	<0.01	0	0	0
hexachlorobenzene	NA	0.010	—	<0.01	0	0	0	0	0	0
heptachlor epoxide	0.3	0.012	4.0	<0.02	0	0	0	0	0	0
chlorpyrifos	0	0.072	<100	<0.37	0	0	0	0	0	0

*Mean concentration of samples containing residues
#AL = percent of FDA Action Limit of the average concentration of residue
ϕRange from limit of detection to concentration reported

Dieldrin was detected in 3 percent of catfish samples and 3 percent of trout samples. Residues of dieldrin in catfish ranged from 0.01 to 0.03 ppm, with an average of 0.019 ppm, or 6.33 percent of the FDA Action Limit of 0.3 ppm. One trout sample contained 0.01 ppm dieldrin, which is 3.3 percent of the Action Limit. There were no dieldrin residues detected in the crayfish samples.

Hexachlorobenzene was detected in 1 percent of the catfish samples for an average of 0.01 ppm. The FDA has not established an Action Limit for hexachlorobenzene in fish. Residues were not detected in trout or crayfish.

Heptachlor epoxide was found in 2 percent of the catfish samples, with a range of 0.01 to 0.02 ppm and an average of 0.012 ppm, or 4 percent of the FDA Action Limit of 0.3 ppm. Heptachlor epoxide was not detected in the trout or crayfish samples.

Chlorpyrifos was detected in 11 percent of the catfish samples and ranged from 0.01 to 0.37 ppm, with an average of 0.072 ppm. Since there is no FDA Action Limit for this currently registered pesticide, all residues are considered violative. Upon finding residues of chlorpyrifos in catfish samples collected from two states, the pesticide was banned from the geographical area where positive residues had been detected and no further chlorpyrifos residues were detected. Trout and crayfish samples had no detectable chlorpyrifos residues.

In Table 8, the residues found in catfish from production sites are compared to those collected from processing sites. In almost all cases, the residues found in catfish tissue from production sites were lower than in fish collected from processing sites. This may be due to the greater loss of body fat that occurs in hand-cleaned and filleted fish than in fish collected from processors.

Table 8. Percent of total catfish samples with detectable residues. Samples were from production and processing sites.

Contaminant	% Producer samples (n=197)	% Processor samples (n=60)
DDT	45.0	87.0
chlordane	1.0	5.0
PCB	5.6	11.6
dieldrin	1.5	6.6
hexachlorobenzene	0	3.3
heptachlor epoxide	1.0	5.0
chlorpyrifos	6.6	3.9

Conclusions

This study shows the importance of differentiating aquaculture products from sportfish when warning consumers about the dangers of consuming contaminated fish. The metals analyzed in the study, many of which are essential micronutrients, are found at lower concentrations in aquaculture products than in sportfish. Most of the organic residues detected were below the action limits established by the FDA. Chlorpyrifos is an exception, since there is no established level. However, due to the sporadic nature of environmental contaminants, this study demonstrates the need for the aquaculture industry to incorporate pesticide residue testing into quality assurance programs.

Objective 4: Determine the Influence of Cooking Processes on Pesticide and Antibiotic Residues

The purpose of the study was to determine the effects of cooking on residues in channel catfish fed a diet containing dieldrin, DDE, chlordane, toxaphene, ormetoprim (OMP), sulfadimethoxine (SDM) or oxytetracycline (OTC). Four cooking methods were compared—frying, frying after infusion with polyphosphate solution, baking and smoking.

Methods

Channel catfish were fed a diet containing one of the test pesticides. The diets were fed at 1 percent body weight for 25 days, then at 0.5 percent for 13 days. Concentrations of pesticides in the feed were: dieldrin, 4 ppm; p,p'-DDE, 5 ppm; chlordane, 2 ppm; and toxaphene, 2 ppm. Fish (N = 24) were harvested and filleted. One fillet was kept raw and the other was cooked. Residue concentrations were determined using a gas chromatograph with an electron capture detector.

To dose catfish with OMP and SDM (Romet-30), 14 catfish were placed in each of seven tanks and fed either a control diet or a diet containing Romet-30 at 25.0, 50.0 or 100.0 mg/kg fish weight. One tank was used as the control and the other six were equally divided among the medicated diets. Catfish were fed the diets for 5 days at 2 percent body weight and then held off feed for 18 hours before sampling. Samples were prepared using one of the four cooking methods and stored at -23 ^{o}C for later analysis as described in Objective 1.

To dose catfish with oxytetracycline, 14 fish (0.87 kg body weight) were placed in each of six concrete tanks with two tanks used for each of the three dose levels. Feeds with 1.88, 3.75 or 7.50 g OTC/kg feed were fed to fish at 2 percent body weight daily for 10 days. If all feed was consumed, fish received 37.5, 75.0 or 150.0 mg OTC/kg body weight per day. Fish were starved for 18 hours before sampling. The samples were prepared using one of the four cooking methods and stored at -23 ^{o}C for later analysis.

Some fillets were breaded and fried at 190 ^{o}C for 7 to 10 minutes until golden brown. A second group of fillets was injected with a 6 percent polyphosphate solution (to a maximum of 10 percent by weight), stored at 23 ^{o}C for 4 weeks, then breaded and fried using the deep fat method described above. A third group was baked at 190 ^{o}C for approximately 45 minutes. The fourth group was soaked in a 25 percent NaCl solution for 1 hour at 10 ^{o}C, then air-dried, and then smoked for 2 hours (smoke was applied for 30 minutes).

Additional information on this study is available in:

Khanna, N., C.R. Santerre, D. Xu and Y.W. Huang. 1997. Changes in Dieldrin and p,p'-DDE Residues Following Cooking of Channel Catfish. *Journal of Food Protection* 60(3):300-304.

Santerre, C.R., R. Ingram, D.H. Xu, G.W. Lewis and L.G. Lane. 2000. Chlordane and Toxaphene Residues Following Cooking of Treated Channel Catfish Fillets, *Journal of Food Protection* 63(6): 763-767.

Xu, D.H., J.M. Girzzle, W.A. Rogers and C.R. Santerre. 1996. Effects of Cooking on Residues of Ormetoprim and Sulfadimethoxine in the Muscle of Channel Catfish. *Food Research International* 29(3-4):339-344.

Results

Dieldrin, p,p'-DDE, Chlordane and Toxaphene

To account for changes in moisture and fat content during cooking, the concentrations of each residue were calculated on a dry and a fat basis. Frying and smoking reduced dieldrin residues by more than 60 percent when calculated on a dry basis (Table 9) and by more than 55 percent when calculated on a fat basis. Baking did not reduce dieldrin residues as much as the other methods. The four cooking methods reduced p,p'-DDE by 53 to 82 percent on a dry basis and by 39 to 73 percent on a fat basis. Chlordane was reduced by 56 to 86 percent with frying, and by only 9 to 12 percent with smoking

Table 9. Average residues of dieldrin, p,p'-DDE, chlordane and toxaphene before and after cooking catfish (n=6) fillets.

Dry basis	Dieldrin (ppb) Mean ± SD	DDE (ppb) Mean ± SD	Chlordane (ppb) Mean ± SD	Toxaphene (ppm) Mean ± SD
Raw	649 ± 40	533 ± 67	131 ± 48	0.697 ± 0.30
Fried	222 ± 41	251 ± 34	56 ± 26	0.346 ± 0.13
%difference	-65.8	-53	-56	-49
Raw	651 ± 144	433 ± 26	123 ± 31	0.639 ± 0.17
PO_4-injected-fried	251 ± 41	111 ± 35	15 ± 1	0.392 ± 0.18
%difference	-61.4	-74	-86	-40
Raw	627 ± 100	498 ± 73	142 ± 39	0.631 ± 0.19
Baked	321 ± 60	246 ± 48	124 ± 34	0.399 ± 0.12
% difference	-48.8	-51	-12	-35
Raw	623 ± 36	431 ± 100	179 ± 55	0.561 ± 0.10
Smoked	242 ± 61	78 ±20	157 ± 39	0.407 ± 0.14
% difference	-61.5	-82	-9	-24

Fat basis	Dieldrin (ppb) Mean ± SD	DDE (ppb) Mean ± SD	Chlordane (ppb) Mean ± SD	Toxaphene (ppm) Mean ± SD
Raw	3,186 ± 58	2,225 ± 531	4,070 ± 1,800	14.4 ± 5.5
Fried	824 ± 177	1,048 ± 220	560 ± 280	3.3 ± 1.6
% difference	-74	-53	-84	-77
Raw	2,913 ± 95	2,018 ± 365	2,410 ± 610	14.6 ± 3.2
PO_4-injected-fried	1,055 ± 22	617 ± 150	180 ± 30	5.0 ± 2.3
% difference	-64	-69	-92	-65
Raw	2,996 ± 45	2,589 ± 43	3,120 ± 620	14.7 ± 6.9
Baked	1,924 ± 25	1,581 ± 480	2,140 ± 400	6.4 ± 1.7
% difference	-36	-39	-30	-51
Raw	3,392 ± 74	1,498 ± 321	3,060 ± 1,360	13.4 ± 2.1
Smoked	1,532 ± 37	398 ± 110	2,030 ± 840	5.3 ± 1.7
% difference	-55	-73	-33	-59

and baking. On a fat basis, frying reduced chlordane by 84 to 92 percent, while smoking and baking reduced it by 30 to 33 percent. Toxaphene was reduced by 24 to 49 percent by all four cooking methods on a dry basis, and by 51 to 77 percent on a fat basis. Overall, pesticide residues were reduced by 9 to 86 percent on a dry basis and by 33 to 92 percent on a fat basis.

Ormetoprim and Sulfadimethoxine

Fillets injected with polyphosphate had OMP residues 56.6, 40.0 and 79.5 percent lower and SDM levels 36.8, 46.1 and 76.2 percent lower than the corresponding raw fillets (Figs. 2 and 3). OMP residue levels in baked fillets were 17.3, 77.5 and 60.4 percent lower than in the raw fillets. SDM levels in baked fillets were 30.9, 47.0 and 61.9 percent lower than in raw fillets (Fig. 4). Smoked fillets had lower residue levels at 12.5, 66.4 and 52.6 percent, and lower SDM levels at 35.2, 51.8 and 52.7 percent, respectively (Fig. 5). No statistical differences were noted between cooking methods. Cooking significantly lowered residue levels of OMP and SDM in channel catfish fillets. The fish in this study were not subjected to the required 3-day withdrawal from Romet-30 before harvesting; that withdrawal period, in combination with cooking, would give the consumer an additional safety margin.

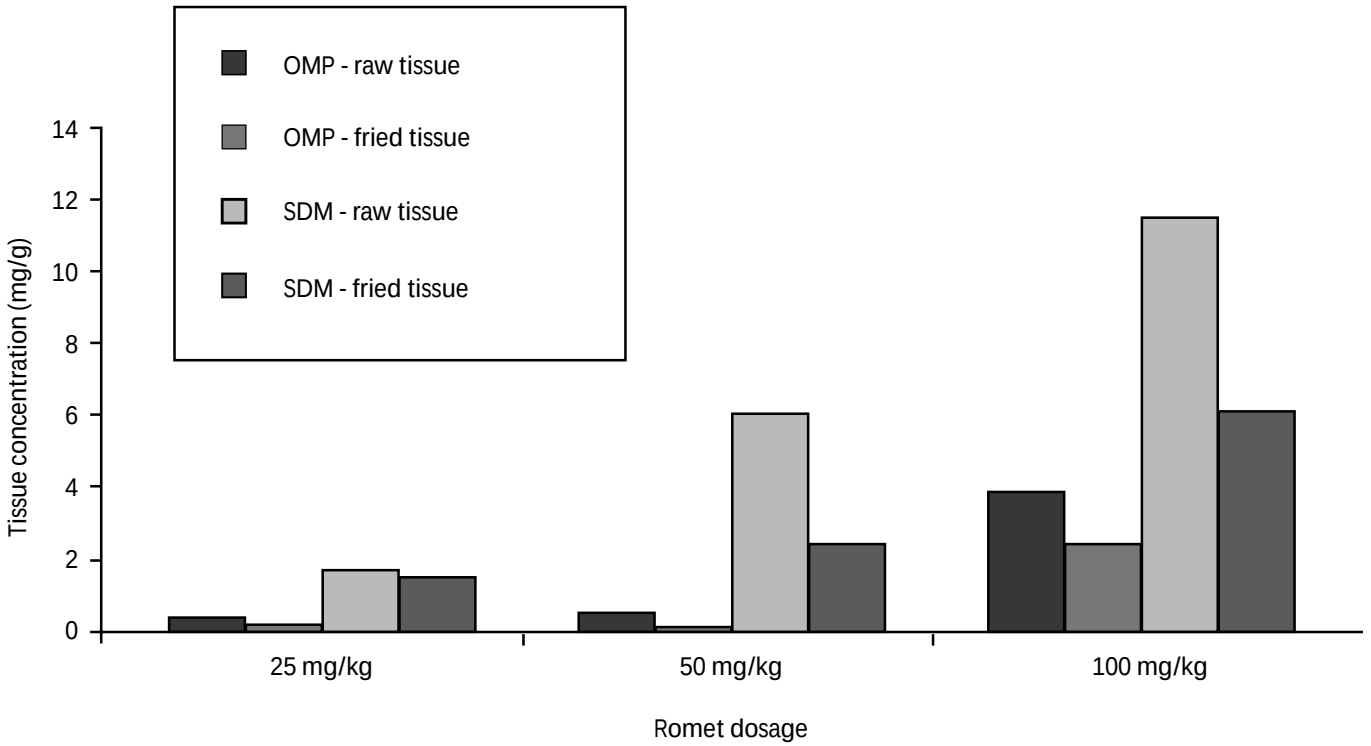

Figure 2. Comparison of ormetoprim (OMP) and sulfadimethoxine (SDM) concentrations in raw and fried fillets from Romet-fed channel catfish.

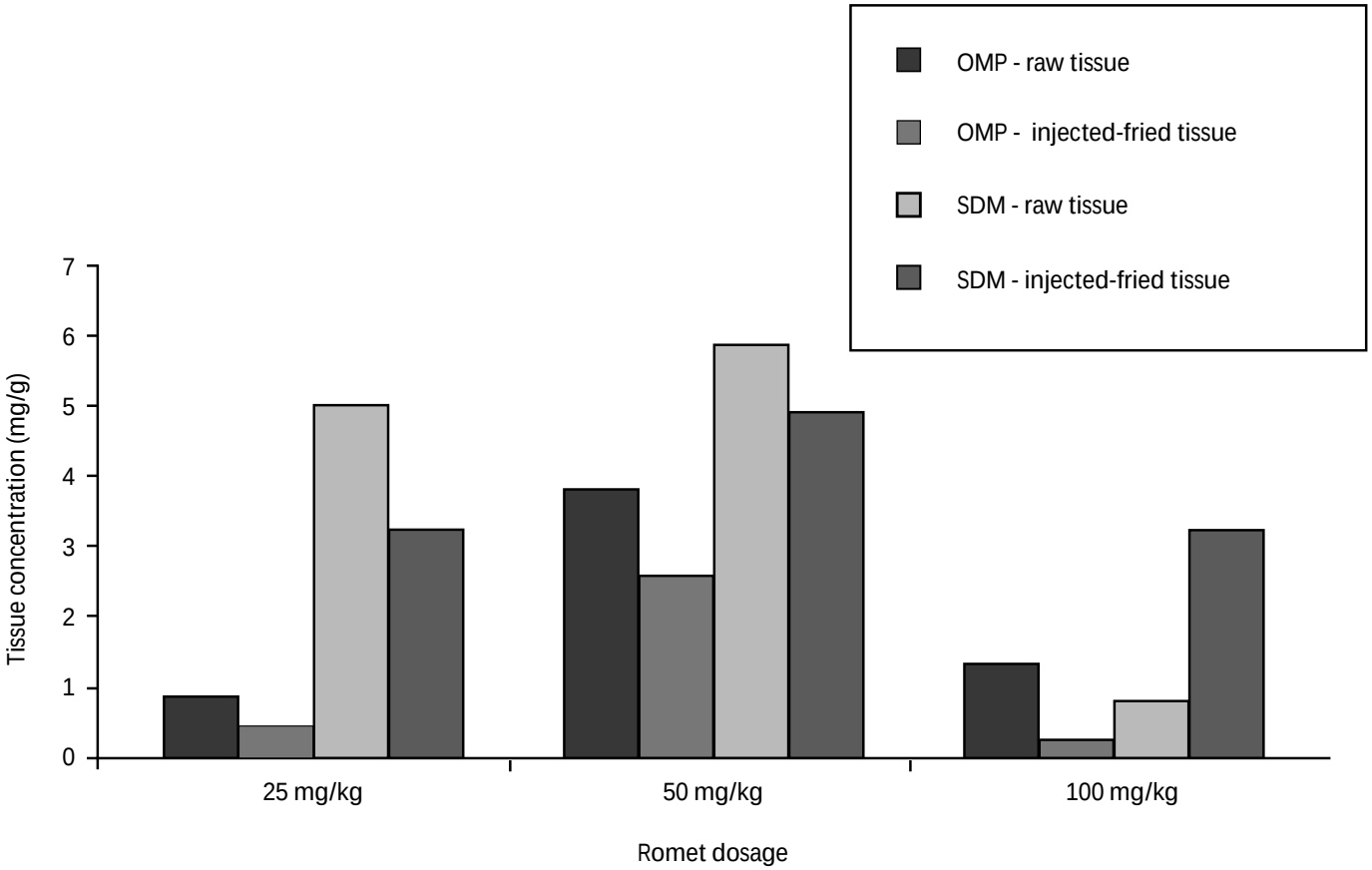

Figure 3. Comparison of ormetoprim (OMP) and sulfadimethoxine (SDM) concentrations in raw and injected-then-fried fillets from Romet-fed channel catfish.

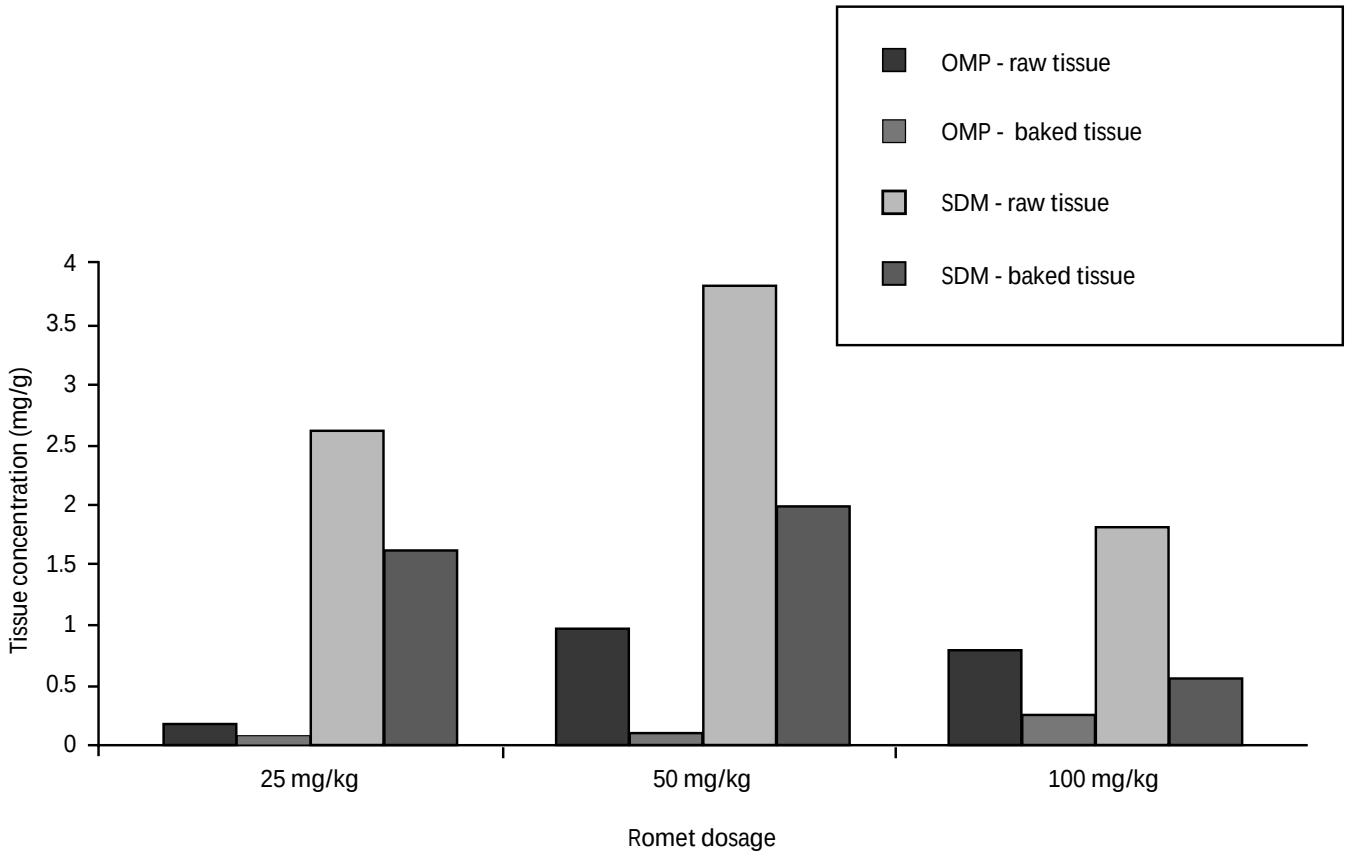

Figure 4. Comparison of ormetoprim (OMP) and sulfadimethoxine (SDM) concentrations in raw and baked fillets from Romet-fed channel catfish.

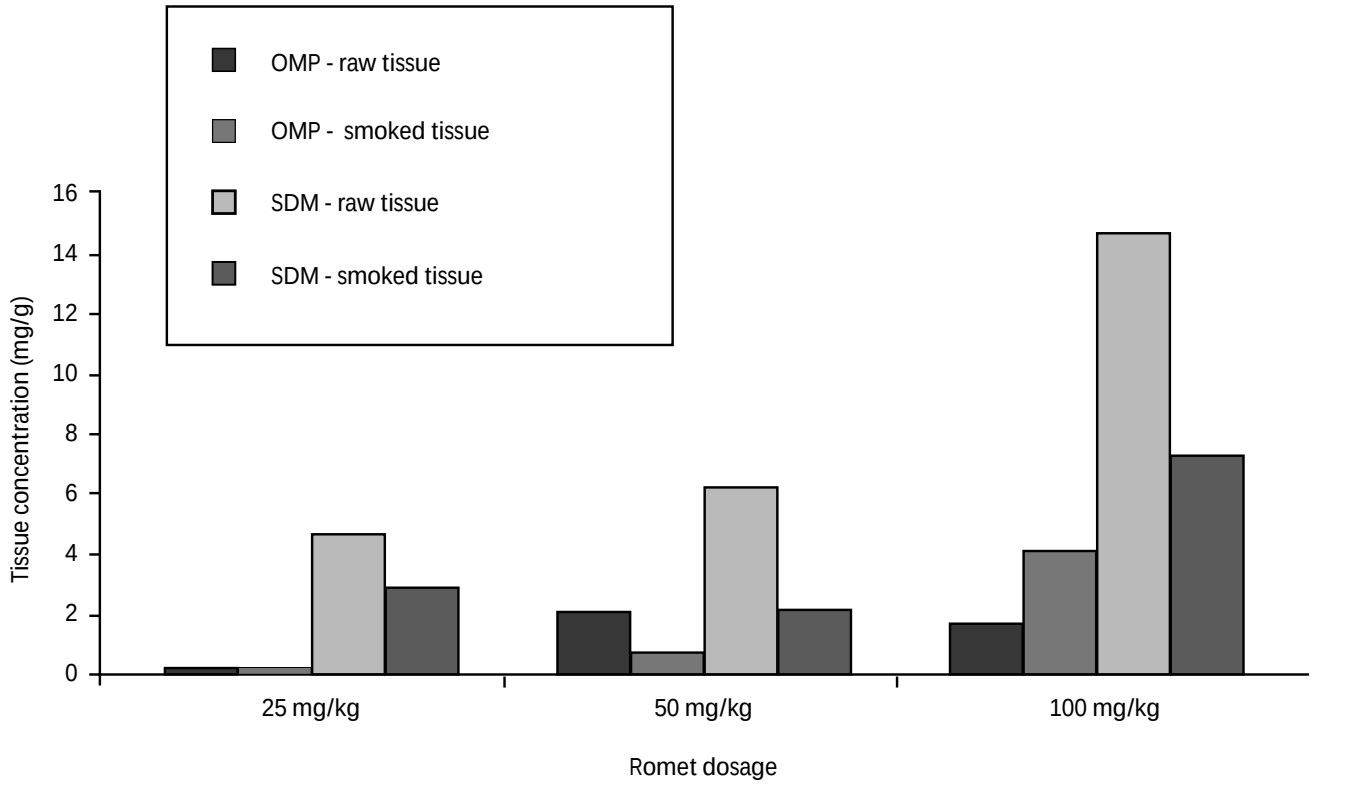

Figure 5. Comparison of ormetoprim (OMP) and sulfadimethoxine (SDM) concentrations in raw and smoked fillets from Romet-fed channel catfish.

Oxytetracycline

While cooking reduced OTC residues, on average the levels were not reduced below the 0.1 ppm tolerance level. Residue levels are found in Table 10. Because of variations in individual fish, results are more difficult to interpret. However, in general, catfish receiving higher dosages of OTC had higher OTC residues. Smoking and baking reduced OTC residues more than frying. Cooking alone does not reduce OTC residues to within the 0.1 ppm tolerance level. In order to ensure safe levels, the OTC dosage regulations and withdrawal times need to be followed.

Table 10. Comparison of liquid chromatography (LC) and capillary electrophoresis (CE) methods for oxytetracycline (OTC) determination on a dry weight basis (n=6).

Fortification level	LC Mean ± SD			CE Mean ± SD		
	37.5 mg/kg	**75.0 mg/kg**	**150.0 mg/kg**	**37.5 mg/kg**	**75.0 mg/kg**	**150.0 mg/kg**
Raw	0.56 ± 0.37	070 ± 0.87	0.99 ± 0.66	0.36 ± 0.34	0.48 ± 0.37	1/67 ± 0.75
Fried	0.21 ± 0.16	0.46 ± 0.37	0.49 ± 0.13	0.16 ± 0.19	0.32 ± 0.23	0.46 ± 0.36
% difference	-63	-34	-51	-56	-33	-72
Raw	0.56 ± 0.40	0.54 ± 0.18	1.59 ± 1.71	0.74 ± 0.75	0.39 ± 0.19	2.38 ± 2.7
Baked	0.14 ± 0.14	0.05 ± 0.05	0.57 ± 0.85	0.05 ± 0.09	0.17 ± 0.17	1.12 ± 1.81
% difference	-75	-91	-64	-93	-56	-53
Raw	0.54 ± 0.61	2.01 ± 1.83	1.07 ± 0.53	0.36 ± 0.58	0.74 ± 0.40	1.50 ± 0.46
Injected-fried	0.18 ± 0.19	0.94 ± 0.99	0.95 ± 0.57	0.25 ± 0.18	0.39 ± 0.33	0.68 ± 0.36
% difference	-67	-53	-11	-31	-47	-55
Raw	0.28 ± 0.23	0.63 ± 0.54	0.89 ± 1.28	0.04 ± 0.05	0.33 ± 0.19	1.69 ± 2.71
Smoked	0.11 ± 0.08	0.20 ± 0.13	0.12 ± 0.15	0.03 ± 0.04	0.08 ± 0.07	0.34 ± 0.28
% difference	-61	-68	-87	-25	-76	-80

Objective 5: Determine the Effects of Antibiotics on Fish Growth

The use of antibiotics as growth enhancers is well established in the poultry and swine industries, although neither of the two antibiotics approved for use in channel catfish production—oxytetracycline (OTC) and Romet-30 (a mixture of sulfadimethoxine and ormetoprim)—are approved for use as growth enhancers. The following study was conducted to determine how the extended use of OTC and Romet-30 affect catfish growth and tissue residue.

Additional information about this study can be obtained from:

S. D. Rawles, A. Kocabas, D.M. Gatlin III, W. X. Du and C. I. Wei, 1998, Dietary Supplementation of Terramycin and Romet-30 Does Not Enhance Growth of Channel Catfish But Does Influence Tissue Residues, *Journal of the World Aquaculture Society* 28:392-401.

Methods

Diets were formulated for two controlled feeding trials. The base diet for Trial 1 used purified ingredients and was formulated with 30 percent crude protein, 3.44 kcal of digestible energy/g and a lipid component to enhance palatability. Six versions of the diet were fed, with either Romet-30 or OTC at 13.8, 25.0 or 50.0 mg/kg of fish per day when fed at 3 percent (dry matter basis) of body weight per day. The Trial 2 diet used 30 percent crude protein from practical ingredients and 2.5 kcal digestible energy/g. This diet was then formulated into six practical diets that contained Romet-30 or OTC alone at 50 or 100 percent, or an equal mixture of both at 50 or 100 percent of the recommended doses. Fingerling channel catfish were conditioned indoors for 2 weeks on the appropriate control diet and then stocked at 10 per aquarium for Trial 1 and 11 per tank for Trial 2. Daily rations were computed at 3 percent of body weight on a dry-matter basis and fed in two equal feedings. The catfish remained on the diets for 8 weeks. Trial 1 fish were then fed the appropriate control diet for an additional 3 weeks and Trial 2 fish were fed the appropriate control diet for 4 weeks. Fish were weighed weekly and the weight gain, feed efficiency and feed allowance were recorded. Samples were collected and shank fillets prepared from each treatment in Trial 1 at 0, 4, 8 and 11 weeks. In Trial 2, samples were collected at 4, 8 and 12 weeks. In both trials, samples were frozen at -17 ^{o}C. Antibiotic residues were determined by liquid chromatography.

Results

After 4 weeks of treatment, fish fed the OTC and control diets had greater weight gain and feed efficiency than those fed Romet-30 diets, although these differences were not significant at 8 weeks. Survival rates were significantly higher in the Romet-30 treatment than in either the control or the OTC treatment. Overall, fish survival in Trial 1 ranged from 93 to 100 percent, with similar results in Trial 2. Fish fed the Romet-30 diet did not perform as well as those fed the control or OTC diets during the first 4 weeks (Table 11). However differences in weight gain were not significantly different in any of the diets after 8 weeks. In Trial 1, mean residue levels of OTC and OMP were positively related to dosage level but were not affected by length of time fed (Table 12). Mean residues of SDM, also positively related to dosage level, were significantly different between the 4-week and 8-week feeding times. Residues of OTC were found in fish fed the 50 or 100 percent OTC diets. Residues of OMP were found in fish fed the 100 percent Romet-30 diet, whereas SDM residues were detected in fish fed all diets. In Trial 2, fish fed the OTC diets at 50 or 100 percent for 4 weeks were below the legal tolerance, whereas residues were above the legal tolerance after 8 weeks of feeding. The OTC and Romet-30 diets at 50 and 100 percent had residue levels above the legal tolerance although mean residue concentrations could not be calculated because of the limited number of samples analyzed. In fish fed the Romet-30 diets, OMP residues were detected only at the 8-week, 100 percent dosage, whereas SDM residues were detected at all dosage levels. The performance of juvenile channel catfish was not enhanced by the use of either

Table 11. Feed efficiency and weight gain from channel catfish fed diets containing oxytetracycline (OTC) or sulfadimethoxine (SDM) and ormetoprim (OMP).

		Semipurified Diets				Practical Diets			
		4 weeks		8 weeks		4 weeks		8 weeks	
		weight gain	feed efficiency	weight gain	feed efficiency	weight gain	feed efficiency	weight gain	feed efficiency
Control	0	125	1.04	311	0.90	33	0.35	94	0.42
OTC	25	116	1.01	249	0.84	39	0.42	103	0.46
	50	119	1.02	284	0.84	46	0.46	135	0.52
	100	121	1.04	300	0.89	22	0.25	87	0.40
SDM/OMP	25	108	0.96	261	0.81	22	0.25	70	0.35
	50	96	0.88	263	0.81	30	0.32	81	0.41
	100	87	0.81	201	0.78	15	0.18	51	0.31

Table 12. Oxytetraclycline (OTC), ormetoprim (OMP) and sulfadimethoxine (SDM) residues in tissue (ppm).

		Semipurified diets		Practical diets	
Feed fortification amount (%)	Antibiotic	4 weeks	8 weeks	4 weeks	8 weeks
0	OTC	0.00	0.00	0.00	0.03
	OMP	0.00	0.00	0.00	0.00
	SDM	0.00	0.00	0.00	0.06
25	OTC	0.06	0.06		
	OMP	0.01	0.03		
	SDM	0.20	0.31		
50	OTC	0.12	0.10	0.07	0.22
	OMP	0.04	0.05	0.00	0.00
	SDM	0.37	0.62	0.07	0.77
100	OTC	0.16	0.22	0.07	0.72
	OMP	0.14	0.17	0.00	0.20
	SDM	0.74	2.16	0.00	1.29

antibiotic and, in fact, performance may have been poorer than that of control fish. Residues of OTC, OMP and SDM increased in a time- and dose-dependent manner to a point where they exceeded the tolerance. A withdrawal from antibiotics for 3 to 4 weeks decreased residues to undetectable levels.

Conclusions

This study shows that using antibiotics as feed additives does not enhance growth and feed efficiency. In fact, growth and feed efficiency decrease when antibiotics are added to the feed. Fish fed the Romet-30 diets had the poorest performance, which may be attributed to the poor palatability of Romet-30. In addition, fish in Trial 1 outperformed fish in Trial 2. This may be attributed to the fact that the practical diets had 1.5 percent added fish oil while the semipurified diets had 10 percent added fish oil. Differences in water temperatures, fish mean weights and energy content of the diets may also affect fish performance.

Residue levels in fish samples increased as a function of either time or dosage levels, except when fish were subjected to a 3- to 4-week withdrawal period. Differences in individual absorption and metabolism may affect residue levels. In this study, individual muscle samples obtained from fish fed at the 100 percent level for 8 weeks had marked differences in residue levels.

Recommendations

Antibiotics

- Producers must observe dosage levels and strictly adhere to withdrawal times for the approved antibiotics.
- Antibiotics should not be used to increase fish growth rate, since data presented here indicate that antibiotics may actually slow fish growth. Overuse of antibiotics may promote the development of resistant pathogens and reduce the number of drugs available to treat fish diseases.
- Now that liquid chromatograhy and capillary electrophoresis methods for the measurement of antibiotic residues in fish tissue have been developed, rapid assays that can be used by processors should be developed to screen fish as they are received for processing. This should be part of the HACCP program to discourage improper use of antibiotics on the farm.
- Funds should be secured to survey aquaculture products for antibiotic residues.

Pesticides

- Educational programs are needed to teach producers the importance of using only approved pesticides in and around ponds.
- Funding should be provided for the development of rapid assays that could be used by processors to screen for pesticides at the point of purchase. Wan et al. (2000, 2001, 2003) validated a rapid assay for measuring chlorpyrifos in fish tissue that can be used by processors. Lasrado et al. (2002) developed a rapid assay for measuring PCB in fish tissue that also can be used by processors. Both assays offer a low-cost, rapid method of screening fish at the point of purchase. Rapid assays are also being developed to measure organic mercury in fish tissue.
- Producers should keep records on feeds (lot numbers and manufacturers' names) because the primary route of exposure to residues is the diet. Should residues be detected at the processing facility, records can be examined to find the source of contamination.
- Additional funding should be found to test for pesticides that are not detected using a multi-residue method.

Metals

- The aquaculture industry should continue to survey fish for mercury, chromium, arsenic and lead. Fish collected during this research had lower levels of these metals than has been reported in sportfish. Continuing to survey these products will help to ensure their success in the marketplace.

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Publications and Presentations

The following publications and presentations were developed as part of this Southern Regional Aquaculture Center project.

Journal Articles

Du, W. X., M. R. Marshall, W. B. Wheeler, M. Mathews, D. Gatlin, S .D. Rawles, D. H. Xu, W. A. Rodgers and C. I. Wei. 1995. Oxytetracycline, sulfadimethoxine, and ormetoprim residues in catfish by LC. *Journal of Food Science* 60(6):1220-1224 & 1227.

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Abstracts or Papers Presented

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Wan, P., C. R. Santerre, E. R. Richter, J. C. Allgood and D. C. Deardorff. 1999. ELISA analysis of chlorpyrifos in catfish. Institute of Food Technologists Annual Meeting, Chicago, IL. July 26. Paper No. 28, Session 50C. (This paper was one of five finalists in the graduate paper competition in the Toxicology and Safety Evaluation Division)

Du, W. X., M. R. Marshall, D.H. Xu, C. R. Santerre and C.I. Wei. 1996. Effect of cooking on oxytetracycline residues in catfish. Presented at the American Chemical Society 212th annual meeting. Orlando, FL, August (AGFD Abstract 48).

Theses or Dissertations

Khanna, N. 1995. Influence of Processing on Residues of Dieldrin and p, p'-DDE in Channel Catfish. M.S. Thesis. University of Georgia.

Wan, P. 2001. Using Solid Phase Extraction (SPE) and Enzyme Linked Immunosorbent Assay (ELISA) to Measure Chlorpyrifos in Catfish Tissue Before and After Fillet Preparation. M.S. Thesis. Purdue University.

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