Oxytetracycline does not cause growth promotion in finfish¹

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ABSTRACT: Until recently, use of antibiotics to enhance terrestrial animal growth performance was a common, U.S. Food and Drug Administration (FDA)-approved, but controversial practice. There are no FDA-approved production claims for antibiotic drug use in fish, but it is a common misconception that antibiotics are widely used for this purpose in U.S. aquaculture. Antibiotics are not thought to be effective growth promoters in fish, but there is little quantitative data available to address whether there are growth-promoting effects that might incentivize the use of antibiotics in this way, despite legal prohibitions. Therefore, this study was conducted to determine if oral administration of oxytetracycline, an antibiotic with known growth-promoting effects in terrestrial livestock, has a similar effect when applied to channel catfish *Ictalurus punctatus*, hybrid striped bass *Morone chrysops* × *M. saxatilis*, Nile tilapia *Oreochromis niloticus*, or rainbow trout *Oncorhynchus mykiss*. Oxytetracycline products with production claims are typically applied at doses substantially lower than the

approved therapeutic doses for the same products. Medication (0, 0.24, or 1.2 g oxytetracycline dihydrate kg⁻¹ feed) and feeding rates (3% BW d⁻¹) were selected to achieve target daily doses of 0, 16, or 80 mg kg⁻¹ fish representing control, subtherapeutic, and therapeutic treatments. Replicate groups of fish (*N* = 4) were fed accordingly for 8 wk. Overall, oral administration of oxytetracycline did not affect survival or promote growth of the selected taxa, with no significant differences observed for weight gain, feed conversion ratio, or specific growth rate (*P* > 0.05 in all cases). Few differences were observed in organosomatic indices and in the frequency of tissue abnormalities; where present, these differences tended to suggest a negative effect of long-term dietary exposure to oxytetracycline. These data demonstrate that there is no benefit to dietary supplementation with oxytetracycline for nontherapeutic purposes in a range of economically important finfish species. As such, our results indicate there is little incentive to misuse oxytetracycline products for purposes of growth promotion in U.S. aquaculture.

Key words: antibiotic, channel catfish, growth promotion, hybrid striped bass, Nile tilapia, rainbow trout

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INTRODUCTION

Antibiotics are a class of antimicrobial drugs used in the treatment and prevention of infectious diseases, principally bacterial infections. Although originally developed for therapeutic applications in humans and animals, growth and/or efficiency promoting effects of antibiotics were first reported in poultry in 1946 and swine in 1950 (Dibner and Richards, 2005). Subsequently, antibiotics became regularly used for nontherapeutic purposes in animal agriculture in the United States (Dibner and Richards, 2005; Landers et al., 2012). The U.S. Food and Drug Administration (FDA) approved the so-called “production claims” for a number of antibiotic drugs in terrestrial livestock, including treatments to increase the rate of weight gain and improve feed efficiency. Prior to the FDA’s efforts beginning 1 January 2017 to “phase out” the use of antibiotics as growth promoters (FDA, 2017), the use of antibiotics to enhance terrestrial animal growth performance was a common, but controversial practice, given concerns regarding their legitimacy in the context of development of antimicrobial resistance (Mellon et al., 2001; Landers et al., 2012). In fact, the practice became so prevalent in the 1990s that by 2001, it was estimated that as much as 84% of antibiotic usage in the United States was for nontherapeutic, production claims (Mellon et al., 2001), with estimates of nontherapeutic antibiotic usage ranging from roughly 3.1 to 24.6 million pounds annually (Landers et al., 2012). There are no FDA-approved production claims for the use of antibiotic drugs as growth promoters in fish, but it is a common misconception that antibiotics are used for this purpose in U.S. aquaculture. Not only are such uses illegal, it is generally understood among fish culturists that antibiotics are not effective growth promoters in fish, thereby removing any incentive to apply antibiotics this way. However, there is very little quantitative data available that unequivocally demonstrate the effect (or lack thereof) of antibiotic administration on growth performance of fish. Therefore, this study was conducted to determine if oral administration of an antibiotic with known growth-promoting effects in terrestrial livestock has a similar effect when applied to representative fish taxa. Specifically, we evaluated the effect of oxytetracycline treatment on the health and growth performance of channel catfish *Ictalurus*

punctatus, hybrid striped bass *Morone chrysops* × *M. saxatilis*, Nile tilapia *Oreochromis niloticus*, and rainbow trout *Oncorhynchus mykiss*.

MATERIALS AND METHODS

All fish husbandry practices and experimental procedures described below were conducted according to the standards of the Southern Illinois University Institutional Animal Care and Use Committee (SIUC IACUC), and experiments with channel catfish, hybrid striped bass, and Nile tilapia were subject to oversight by the SIUC IACUC under Animal Care and Use Protocol # 15-012. The experiment with rainbow trout was not subject to SIUC IACUC oversight, but followed the same animal care and use procedures and adhered to the same animal welfare standards.

Commercially available aquaculture feeds (rainbow trout, EXTR 450, Rangen, Buhl, ID; all other taxa, Aquamax Grower 300, Purina Animal Nutrition, St Louis, MO) appropriate for the selected taxa were used as is (control) or medicated with oxytetracycline dihydrate (OTC) to achieve standard therapeutic (standard OTC) or subtherapeutic (low OTC) doses when fed to fish at a predetermined feeding rate. Oxytetracycline dihydrate products with FDA-approved production claims in terrestrial livestock are typically applied at doses substantially lower than the approved therapeutic doses for the same products (Table 1). An oxytetracycline dihydrate product, Terramycin 200 for Fish (Phibro Animal Health, West Chester, PA), is approved for a number of therapeutic claims in catfish and salmonids at daily doses of 55 to 82.5 mg kg⁻¹ fish (2.5 to 3.75 g 100 lb⁻¹ fish; FDA, 2015). Although this product is not FDA approved for use in all of the taxa evaluated in our study and is not approved for any production claims in fish, we used this range as the basis for determining the therapeutic and subtherapeutic doses we tested. Accordingly, we selected feed medication rates (standard OTC, 1.2 g kg⁻¹ feed; low OTC, 0.24 g kg⁻¹ feed) and feeding rates (3% BW d⁻¹) to target daily doses of 80 mg kg⁻¹ fish (standard OTC) and 16 mg kg⁻¹ fish (low OTC). All medicated feed was prepared using Liquamycin LA-200 (200 mg oxytetracycline mL⁻¹; Zoetis, Florham Park, NJ), an injectable aqueous solution of oxytetracycline dihydrate available over-the-counter for use in cattle and

Table 1. Dosage rates for selected Food and Drug Administration-approved therapeutic (to treat an infectious disease) and production (to promote growth or improve growth efficiency) claims for oxytetracycline dihydrate–medicated feed (Terramycin product line; Phibro Animal Health, West Chester, PA; adapted from [FDA, 2015](#))

Species	Therapeutic claim(s)	Production claim(s)
Chickens	100 to 500 g ton ⁻¹	10 to 50 g ton ⁻¹
Turkeys	100 to 200 g ton ⁻¹	10 to 50 g ton ⁻¹
Swine	20 g ton ⁻¹	10 to 50 g ton ⁻¹
Cattle	0.5 to 2 g head ⁻¹ daily	0.025 to 0.075 g head ⁻¹ daily
Sheep	20 g ton ⁻¹	10 to 20 g ton ⁻¹
Catfish and salmonids	2.5 to 3.75 g 100 lb ⁻¹ fish daily	No production claims are approved for fish

swine. This product is not approved for use in feed for fish or other animals, but was readily available and, given its aqueous form, facilitated homogeneous incorporation of oxytetracycline dihydrate in small batches of medicated feed prepared by top-coating. It is important to stress that Terramycin 200 for Fish is FDA approved for only certain therapeutic purposes in catfish and salmonids, Liquamycin LA-200 is not FDA approved for use in fish whatsoever, and no antibiotics are FDA approved for production purposes in fish. We used Liquamycin LA-200 experimentally for the purpose of hypothesis testing, and all fish were humanely euthanized and disposed of by landfilling at the conclusion of the study. Briefly, medicated feed was prepared by diluting the appropriate amount of Liquamycin LA-200 in tap water (to a total volume of 50 mL kg⁻¹ feed) and applied to pre-weighed feed pellets in a rotating drum concrete mixer intended for home use. Pellets were mixed until the liquid was absorbed (~2 min), then oil was added [vegetable (soybean) oil for rainbow trout feeds, menhaden fish oil for all other feeds; 5 g kg⁻¹ feed in all cases], and the feed was mixed further until the additional liquid was absorbed (~2 min). The top-coated feed was dried overnight in a food dehydrator (Harvest Saver R-5A, Commercial Dehydrator Systems, Eugene, OR) to remove moisture and prevent molding. To ensure all feeds had the same nutrient composition and quality, unmedicated control feeds were similarly top-coated with water and oil and dried.

Experiments with channel catfish (July–September 2015), hybrid striped bass (September–November 2015), and Nile tilapia (April–June 2016) were conducted at the Southern Illinois University Center for Fisheries, Aquaculture, and Aquatic Sciences (Carbondale, IL). Juvenile

channel catfish (5.4 ± 0.8 g initial individual weight, mean $\pm$ SE; Osage Catfisheries, Osage Beach, MO), hybrid striped bass (27.0 ± 0.2 g; Keo Fish Farm Inc., Keo, AR), or Nile tilapia (53.5 ± 0.6 ; Americulture Inc., Animas, NM) were stocked in a recirculation system (20 fish per tank for Nile tilapia, 10 fish per tank for all other taxa) comprising 12, 150-L tanks and provided with continuous aeration and mechanical and biological filtration. Dietary treatments were randomly assigned to quadruplicate aquaria ($N = 4$) and offered once daily at a rate of 3% of BW, with rations adjusted every 2 wk based on group weights. Water temperature and dissolved oxygen were monitored daily (YSI 550 temperature-oxygen meter; Yellow Springs Instruments, Yellow Spring, OH). Water pH (pHep; Hanna Instruments, Woonsocket, RI), total ammonia nitrogen, nitrite nitrogen, nitrate nitrogen, and alkalinity were measured weekly (Hach DR 2800 portable spectrophotometer; Hach Company, Loveland, CO). Throughout each trial, water quality was maintained within ranges suitable for culturing the appropriate taxon ([Table 2](#)).

The experiment with rainbow trout (August–October 2017) was conducted at the Idaho Department of Fish and Game Eagle Fish Health Laboratory (Eagle, ID). Juvenile rainbow trout (33.8 ± 0.3 g; Hayspur Hatchery Rainbow Trout stock, Idaho Department of Fish and Game, Bellevue, ID) were stocked in a recirculation system, 10 fish per tank, comprising 12, 150-L tanks and provided with continuous aeration, mechanical, and biological filtration. Dietary treatments, water temperature, and water pH were monitored as described above for the other experiments.

After 8 wk, fish were counted and group weighed by tank to determine production performance according to the following metrics:

$$\text{Weight gain (\%)} = 100 \times \frac{\text{Final body weight} - \text{initial body weight}}{\text{Initial body weight}}$$

Specific growth rate

$$(\text{SGR, \% body weight/d}) = 100 \times \frac{\text{Log}_e(\text{final body weight}) - \text{Log}_e(\text{initial body weight})}{\text{Day of feeding}}$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Average individual feed consumption (as-fed basis)}}{\text{Final body weight} - \text{initial body weight}}$$

Feed intake

$$(\text{FI, \%body weight/d}) = 100 \times \frac{\text{Total dry matter intake} / (\text{initial body weight} \times \text{final body weight})^{0.5}}{\text{day of feeding}}$$

Four fish from each tank were arbitrarily selected and euthanized via overdose of tricaine methanesulfonate (Tricaine-S, Western Chemical, Ferndale, WA; $\geq 200 \text{ mg L}^{-1}$ in culture water for a period of 10 min after cessation of opercular movement) for individual weighing and health evaluation. Euthanized fish were subsequently necropsied to assess external (i.e., body surface, fins, gills, and eyes) and internal (i.e., liver, kidney, spleen, adipose tissue, gall bladder, alimentary canal, and musculature) tissues and structures. Evaluators were blind with respect to treatment assignments, and each fish was assessed by a single evaluator. Appearance was documented using qualitative terms to describe abnormal color (i.e., pale, mottled), size (i.e., enlarged), or condition (i.e., eroded, granulated, hemorrhagic) or to record the presence of lesions. After necropsy, liver and visceral masses were weighed to determine organosomatic indices using the following equations:

Hepatosomatic index

$$(\text{HSI}) = 100 \times \left(\frac{\text{Liver weight}}{\text{Whole body weight}} \right)$$

Viscerosomatic index

$$(\text{VSI}) = 100 \times \left(\frac{\text{Total viscera weight}}{\text{Whole body weight}} \right)$$

All production performance data were analyzed by 1-way ANOVA (PROC GLIMMIX) with tanks representing experimental units ($N = 4$) using SAS 9.4 (SAS Institute, Cary, NC). When omnibus tests indicated a significant treatment effect, Tukey's honest significant difference tests were used to determine the significance of differences between treatments. Tissue appearance data were coded as "normal" or "abnormal," and the dichotomized data were analyzed with a chi-square test (PROC

FREQ) for differences in the frequency of normal vs. abnormal observations between the treatments. In all cases, differences were considered significant at critical values of <0.05 .

RESULTS

Overall, oral administration of oxytetracycline did not promote growth and did not affect survival rates of the selected taxa (Table 3). More specifically, none of the production performance parameters assessed were significantly influenced by dietary oxytetracycline inclusion, except HSI hybrid striped bass. In the latter case, fish fed the low OTC feed exhibited higher HSI values than those fed the control feed. Although not statistically significant, small numeric reductions in performance were observed in channel catfish, with fish fed the low OTC and standard OTC feeds exhibiting lower weight gain (635 to $607 \pm 23\%$) and SGR (3.56 to $3.49 \pm 0.00\% \text{ BW d}^{-1}$) in comparison with fish fed the control feed (weight gain = $652 \pm 23\%$, SGR = $3.60 \pm 0.00\% \text{ BW d}^{-1}$). Similarly, Nile tilapia fed the low OTC feed displayed statistically nonsignificant, numerical reductions in weight gain ($349 \pm 18\%$), SGR ($2.73 \pm 0.1\% \text{ BW d}^{-1}$), FCR (0.97 ± 0.1), and FI ($2.91 \pm 0.1\% \text{ BW d}^{-1}$) in comparison with those fed the Control feed (weight gain = $376 \pm 18\%$, SGR = $2.84 \pm 0.1\% \text{ BW d}^{-1}$, FCR = 0.92 ± 0.1 , feed intake = $2.87 \pm 0.1\% \text{ BW d}^{-1}$), though the performance of fish fed the standard OTC feed was more numerically comparable to the Control treatment (weight gain = $363 \pm 18\%$, SGR = $2.78 \pm 0.1\% \text{ BW d}^{-1}$, FCR = 0.93 ± 0.1 , feed intake = $2.87 \pm 0.1\% \text{ BW d}^{-1}$). Although there were no statistically significant treatment effects on production performance of rainbow trout, fish fed the control feed had numerically reduced weight gain ($244 \pm 15\%$) and SGR ($2.25 \pm 0.08\% \text{ BW d}^{-1}$) compared with those fed the low OTC (weight

gain = $259 \pm 15\%$, SGR = $2.32 \pm 0.08\%$ BW d⁻¹) or standard OTC feeds (weight gain = $255 \pm 15\%$, SGR = $2.30 \pm 0.08\%$ BW d⁻¹), and fish fed the low OTC feed exhibited a slight numeric reduction in FCR (1.26 ± 0.05) in comparison with those fed the other feeds (1.30 ± 0.05 for both control and standard OTC groups). Feed intake was marginally, but not statistically, reduced among fish fed the standard OTC feed ($3.05 \pm 0.04\%$ BW d⁻¹) in comparison with both the control feed and low OTC feed ($3.07 \pm 0.04\%$ BW d⁻¹).

Table 2. Water quality observed during feeding trials conducted to assess performance of channel catfish, hybrid striped bass, Nile tilapia, and rainbow trout fed commercially available diets or the same diets medicated with oxytetracycline dihydrate to achieve a standard therapeutic dose (standard OTC, 80 mg kg⁻¹ fish daily) or subtherapeutic dose (low OTC, 16 mg kg⁻¹ fish daily) of oxytetracycline

Taxon and parameter	
Channel catfish	
Temperature (°C)	26.8 ± 0.2
Dissolved oxygen (mg L ⁻¹)	6.33 ± 0.75
Total ammonia nitrogen (mg L ⁻¹)	0.039 ± 0.00
Nitrite nitrogen (mg L ⁻¹)	0.03 ± 0.01
Nitrate nitrogen (mg L ⁻¹)	29.71 ± 14.09
pH	8.09 ± 0.20
Alkalinity (mg L ⁻¹)	136 ± 39
Hybrid striped bass	
Temperature (°C)	26.5 ± 0.2
Dissolved oxygen (mg L ⁻¹)	6.23 ± 0.35
Total ammonia nitrogen (mg L ⁻¹)	0.045 ± 0.01
Nitrite nitrogen (mg L ⁻¹)	0.03 ± 0.01
Nitrate nitrogen (mg L ⁻¹)	55.43 ± 5.92
pH	7.87 ± 0.16
Alkalinity (mg L ⁻¹)	201 ± 69
Nile tilapia	
Temperature (°C)	28.8 ± 0.6
Dissolved oxygen (mg L ⁻¹)	5.62 ± 0.87
Total ammonia nitrogen (mg L ⁻¹)	0.097 ± 0.09
Nitrite nitrogen (mg L ⁻¹)	0.11 ± 0.11
Nitrate nitrogen (mg L ⁻¹)	46.23 ± 8.70
pH	6.97 ± 0.33
Alkalinity (mg L ⁻¹)	165 ± 102
Rainbow trout	
Temperature (°C)	14.0 ± 1.1
Dissolved oxygen (mg L ⁻¹)	7.93 ± 0.23
Total ammonia nitrogen (mg L ⁻¹)	0.027 ± 0.01
Nitrite nitrogen (mg L ⁻¹)	0.004 ± 0.001
Nitrate nitrogen (mg L ⁻¹)	0.258 ± 0.066
pH	7.33 ± 0.14
Alkalinity (mg L ⁻¹)	75 ± 5

Values represent means ± SD.

External and internal tissues appeared to be largely unaffected by oral administration of oxytetracycline (Table 4). Tissue appearance did not vary among the dietary treatments in any of the selected taxa, except for the skin/body surface in channel catfish. Epithelial hemorrhaging and irregularly colored skin were significantly more frequent among fish fed the low OTC feed in comparison with those fed the control feed; these abnormalities were also numerically, but not statistically, more common among fish fed the standard OTC feed. The frequency of skin/body surface abnormalities was also numerically, but not statistically, elevated among Nile tilapia fed the oxytetracycline-medicated feeds. Although other abnormalities were observed with relatively high frequencies (e.g., clubbed gills, eroded or deformed fins, irregularly colored liver tissue), these were relatively mild, not associated with the dietary treatments, and commonly observed in intensively cultured fish.

DISCUSSION

Tetracyclines block protein synthesis by preventing the binding of tRNA to the mRNA ribosome complex (Chopra and Roberts, 2001). They are widely used because of their safety and efficacy against a broad spectrum of bacteria (Chopra and Roberts, 2001), viruses (Topno et al., 2016), protozoans, and helminths (Chopra and Roberts, 2001), and preliminary research suggests anti-inflammatory (Weinberg, 2005; Tilakaratne and Soory, 2014), antiapoptotic (Orsucci et al., 2009; Garrido-Mesa et al., 2013), and neuroprotective properties (Domercq and Matute, 2004; Chukwudi, 2016). Tetracyclines were also commonly administered subtherapeutically to improve growth rates and feed conversion efficacy in agriculture (Dibner and Richards, 2005; Hao et al., 2014). Although the use of antibiotics as growth promoters is well established, the mechanisms by which these antibiotics at subtherapeutic levels act to promote growth and efficiency are still under investigation. As tetracyclines in general are poorly absorbed from the gastrointestinal tract, Agwuh and MacGowan (2006) focus has generally been on their effects in the intestines and on associated bacteria (Dibner and Richards, 2005). As reviewed by Gaskins et al. (2002), Dibner and Richards (2005), and Niewold (2007), the mechanisms have classically been broken down into 1) reducing subclinical infections, 2) reducing generation of harmful bacterial metabolites in the intestines, 3) sparing nutrient use by bacteria, and 3) increasing absorptive efficiency

Table 3. Production performance of channel catfish, hybrid striped bass, Nile tilapia, and rainbow trout fed commercially available diets or the same diets medicated with oxytetracycline dihydrate to achieve a standard therapeutic dose (standard OTC, 80 mg kg⁻¹ fish daily) or subtherapeutic dose (low OTC, 16 mg kg⁻¹ fish daily) of oxytetracycline

Taxon and parameter	Control	Low OTC	Standard OTC	Pooled SE	<i>P</i> value
Channel catfish					
Survival (%)	100	100	100	—	—
Initial weight (g)	5.4	5.4	5.5	0.4	0.767
Final weight (g)	41.0	40.0	38.7	1.3	0.246
Weight gain (%)	652	635	607	23	0.201
SGR ¹ (% BW d ⁻¹)	3.60	3.56	3.49	0.0	0.197
FCR ² (as-fed basis)	0.60	0.61	0.63	0.0	0.281
Feed intake (% BW d ⁻¹)	2.55	2.56	2.57	0.0	0.905
HSI ³	1.0	1.1	1.0	0.1	0.972
VSI ⁴	8.0	8.1	7.7	0.4	0.579
Hybrid striped bass					
Survival (%)	97	100	100	1.4	0.405
Initial weight (g)	26.9	27.1	27.0	0.2	0.790
Final weight (g)	85.6	87.0	87.2	4.8	0.937
Weight gain (%)	218	221	223	18	0.952
SGR (% BW d ⁻¹)	2.05	2.08	2.09	0.1	0.941
FCR (as-fed basis)	1.25	1.23	1.22	0.1	0.944
Feed Intake (% BW d ⁻¹)	2.70	2.69	2.68	0.1	0.972
HSI	2.8 ^b	3.2 ^a	2.9 ^{ab}	0.1	0.021
VSI	13.7	14.3	13.8	0.4	0.243
Nile tilapia					
Survival (%)	97	100	97	2.0	0.622
Initial weight (g)	53.1	53.5	54.0	0.6	0.391
Final weight (g)	252.8	240.5	249.9	7.2	0.254
Weight gain (%)	376	349	363	12	0.122
SGR (% BW d ⁻¹)	2.84	2.73	2.78	0.0	0.119
FCR (as-fed basis)	0.92	0.97	0.93	0.0	0.120
Feed Intake (% BW d ⁻¹)	2.87	2.91	2.87	0.0	0.360
HSI	2.0	1.9	1.9	0.1	0.728
VSI	8.7	7.9	8.5	0.7	0.518
Rainbow trout					
Survival (%)	100	100	100	—	—
Initial weight (g)	33.8	33.7	33.9	0.3	0.866
Final weight (g)	116.3	121.0	120.5	5.8	0.684
Weight gain (%)	244	259	255	15	0.615
SGR (% BW d ⁻¹)	2.25	2.32	2.30	0.08	0.619
FCR (as-fed basis)	1.30	1.26	1.30	0.05	0.719
Feed intake (% BW d ⁻¹)	3.07	3.07	3.05	0.04	0.862
HSI	1.3	1.5	1.4	0.1	0.609
VSI	12.5	13.7	12.5	0.5	0.225

Values represent least-square means; pooled SE values and *P* values resulting from 1-way ANOVA tests are also provided. For parameters exhibiting significant treatment effects, means with common letter labels are not significantly different; the absence of letter labels indicates the absence of a significant treatment effect (*P* > 0.05).

¹Specific growth rate.

²Feed conversion ratio.

³Hepatosomatic index.

⁴Viscerosomatic index.

by thinning the intestinal wall. However, an alternate theory is that the main mechanism of action of oxytetracycline as a growth promoter is driven by its anti-inflammatory effects (Niewold, 2007;

Khadem et al., 2014). For example, there was a significant decrease in acute phase proteins and markers of inflammation with a concomitant increase in weight gain in weanling pigs fed oxytetracycline

(Soler et al., 2016). Similarly, supplementation of broiler chicken diets with oxytetracycline from 0- to 35-d posthatch led to increased feed intake and feed efficiency and decreased intestinal inflammation as evidenced by downregulation of the jejunal expression of inducible nitric oxide synthase (Khadem et al., 2014). In these cases, the reduction in inflammation is believed to result in higher feed intake, reduced need for acute protein synthesis, and increased nutrient availability for tissue development (Soler et al., 2016). Regardless of whether antibiotic growth promoters are having a direct effect on the microbial populations in the gastrointestinal tract or working as an anti-inflammatory agent, there is the common theme of redirecting nutrients and energy from protective, but energetically costly, mechanisms toward tissue synthesis and somatic growth. However, oxytetracycline appears to have the opposite effect in fish, causing oxidative

stress and immunosuppression (Yonar et al., 2011; Yonar, 2012). Although reducing energetic expenditure for immune function would be consistent with at least one proposed mechanism for growth promotion, the energetic cost of coping with significant oxidative stress may negate and perhaps overwhelm any energetic savings associated with immunosuppression. The relationship between oxytetracycline and oxidative stress in fish may explain the lack of a consistent growth-promoting effect of this antibiotic in aquatic livestock.

The literature describing possible growth-promoting effects of antibiotics in fish is scant, and the few published studies report conflicting results. Channel catfish with active *Edwardsiella ictaluri* infections grew more when offered feed medicated with sulfadimethoxine/ormetoprim according to some, but not all feeding regimens (Wise and Johnson, 1998). Given the disease state of these

Table 4. Frequency (%) of normal and abnormal tissue appearance in channel catfish, hybrid striped bass, Nile tilapia, and rainbow trout fed commercially available diets or the same diets medicated with oxytetracycline to achieve a standard therapeutic dose (standard OTC, 80 mg kg⁻¹ fish daily) or subtherapeutic dose (low OTC, 16 mg kg⁻¹ fish daily) of oxytetracycline

Taxon and tissue	χ^2 (P-value)	Control	Low OTC	Standard OTC	Nature (#) of abnormalities
Channel Catfish					
Skin and body surface	8.18 (0.017)				H _{body} (14), I _{Cbody} (12), H _{fin} (11), Df _{fin} (7), Er _{fin} (5), I _{Cfin} (1)
Eyes	1.03 (0.310)				CI (1)
Gills	2.37 (0.306)				Cb (30), Er (14)
Muscle	0 (NA)
Alimentary canal	0.82 (0.664)				Ic (6), G (2), P (2), L (1), H (1)
Liver	0.58 (0.748)				M (10), Ic (8), H (3), P (2), G (1)
Gall bladder	0 (NA)
Kidney	1.03 (0.310)				H (1)
Spleen	0.25 (0.881)				G (9), Ic (1)
Intraperitoneal adipose tissue	0 (NA)
Hybrid Striped Bass					
Skin and body surface	0.24 (0.889)				E _{fin} (10)
Eyes	5.74 (0.057)				CI (8), H (2)
Gills	4.80 (0.091)				Cb (16), Ic (5)
Muscle	0 (NA)
Alimentary canal	2.13 (0.144)				H (2)
Liver	0 (NA)				M (30), P (18)
Gall bladder	2.44 (0.296)				P (11), Ic (5)
Kidney	0 (NA)
Spleen	0 (NA)
Intraperitoneal adipose tissue	0 (NA)

Continued

Table 4. Continued

Taxon and tissue	P-value	Control	Low OTC	Standard OTC	Nature (#) of abnormalities
Nile Tilapia					
Skin and body surface	4.01 (0.135)				Er _{fin} (35), Df _{fin} (3)
Eyes	0.25 (0.881)				Cl (10)
Gills	0 (NA)				
Muscle	0 (NA)				
Alimentary canal	1.03 (0.310)				L (1)
Liver	0.20 (0.904)				P (25), M (8), L (3), Ic (3), Fa (2)
Gall bladder	1.03 (0.310)				Sm (1)
Kidney	0 (NA)				
Spleen	1.03 (0.310)				EI (1)
Intraperitoneal adipose tissue	0 (NA)				
Rainbow Trout					
Skin and body surface	0 (NA)				H _{fin} (27), H _{fin} (2), Er _{fin} (48), Er _{rostrum} (4), Er _{mandible} (1), Df _{mandible} (2), DF _{operculum} (7), Tr _{rostrum} (5), S (1)
Eyes	0 (NA)				
Gills	0.15				Er (4)
Muscle	0 (NA)				
Alimentary canal	0 (NA)				
Liver	0 (NA)				
Gall bladder	0.59				EI (1), Sm (1)
Kidney	0 (NA)				
Spleen	0 (NA)				
Intraperitoneal adipose tissue	0 (NA)				

Cb = clubbed, Cl = cloudy, Df = deformed, EI = enlarged, Er = eroded, Fa = fatty, G = granulomatous, H = hemorrhagic, Ic = irregular color, L = lesions, M = mottled, P = pale, Sm = small, Tr = truncated, S = scoliosis

For each treatment, filled boxes represent the frequencies of normal (gray fill) and abnormal (white fill) tissue. For tissues exhibiting abnormal appearance, the nature (score codes, see footnotes for details) and number (in parentheses) of these observations are also provided. A total of 16 fish were examined for each treatment; note that observations of abnormalities may sum to a number greater than 16 as some individuals exhibited multiple abnormalities. *P* values indicating significant differences in frequency distributions are provided. For datasets in which no variation was observed, the χ^2 value is 0 and no *P* value was calculated (NA = not applicable).

*Frequency distributions for experimental treatments that are significantly different from that of the control treatment.

fish and the generally reduced feeding behavior of sick fish, it is most reasonable to conclude that the apparent effect of antibiotic-medicated feed on growth of channel catfish observed by Wise and Johnson had more to do with treatment and resolution of the bacterial infection than growth promotion. When other investigators have offered antibiotic-medicated feeds to healthy channel catfish the results have been mixed: Rawles et al. (1997) reported no effects of oxytetracycline or sulfadimethoxine/ormetoprim exposure on growth performance, whereas Sanchez-Martínez et al. (2008) reported a modest but significant increase in weight gain and condition of fish fed oxytetracycline-medicated feed. Feeds medicated with florfenicol or oxytetracycline improved growth and feed conversion ratio in Nile tilapia, though florfenicol was a more effective growth promoter

than oxytetracycline and did not cause histological anomalies to the extent of the latter (see below, Reda et al., 2013). Oxytetracycline reportedly reduces feed palatability in some finfish, indirectly affecting growth performance. For example, Atlantic salmon fed oxytetracycline-medicated feed exhibited numeric reductions in voluntary feed intake for the first few weeks of a 9-wk feeding trial (Toften and Jobling, 1997a). This effect waned as the trial progressed and fish grew accustomed to the taste of the medicated feed, but final weight was nonetheless reduced as a result of the lost growth potential at the beginning of the trial. These authors reported long-term feeding of oxytetracycline imposed other growth-suppressive effects beyond palatability: even when a palatant (squid extract) was added to mask the odor/flavor of oxytetracycline, feed digestibility and growth

were still impaired as a result of dietary oxytetracycline exposure (Toften and Jobling, 1997a). Similar, digestibility-related effects of long-term oxytetracycline feeding were observed in Arctic Charr *Salvelinus alpinus* (Toften and Jobling, 1997b), adding strength to the argument that oxytetracycline exposure may actually depress fish performance over time. Exposure to oxytetracycline also appears to suppress some elements of the immune system in fish, while possibly stimulating others (Yonar et al., 2011; Guardiola et al., 2012; Yonar, 2012); although the cumulative effects on the immune system are unclear, these results suggest that immunological interference is at least a cause for concern.

It is somewhat puzzling that HSI values were elevated in hybrid striped bass fed the low OTC feed. Increasing hepatic mass can be associated with excess energy intake or toxicity, but in the absence of histological information, we cannot say which is the case for hybrid striped bass in the present study. The fact that we did not observe increased HSI values in hybrid striped bass fed the standard OTC feed (or any of the other taxa fed oxytetracycline-medicated feeds) would seem to argue against toxicity, but the results presented herein do not support a definitive conclusion regarding the possible effects of long-term oxytetracycline exposure on hepatic tissues or their function. However, others have reported histological abnormalities in the renal and hepatic tissues of Nile tilapia fed oxytetracycline- and florfenicol-medicated feeds for extended periods of time, with more severe lesions among fish exposed to oxytetracycline, suggesting that long-term exposure to these antibiotics negatively affects these organs and their function (Reda et al., 2013). The causal basis for more skin/body surface abnormalities among channel catfish fed the low OTC feed, but not standard OTC feed, is also unclear. It is possible that scaleless fish, like channel catfish, may be more sensitive to epithelial effects of antibiotics or other agents, but as with the hybrid striped bass HSI data, the results we observed are not fully consistent with a toxic or similar effect (i.e., the effects were not exaggerated among fish fed the standard OTC feed). Although the information available is not exhaustive, the weight of evidence reported in the literature and in the present study suggests that the effects of antibiotics on growth, efficiency, and condition/health in fish are minor, but generally negative when present.

Of the antibiotics that are FDA approved for therapeutic use in aquaculture, ormetoprim/

sulfadimethoxine use in U.S. aquaculture was estimated for 2001 to 2003 to be 17,340 kg, whereas oxytetracycline usage for the same period was estimated at 11,167 kg (Viola and DeVincent, 2006; aquaculture production during this time was ~0.5 million metric tons; FAO, 2018). To put this in perspective, the same source estimated that the United States used 10 million kg of antimicrobial products per year in companion animals and livestock over the same time period (red meat production during this time was ~20.8 million metric tons, egg production was ~85 billion eggs, and broiler production was ~18.9 million metric tons; USDA, 2002a,b). Although this demonstrates the quantitatively low use of antibiotic compounds by the aquaculture industry, consumers remain inordinately concerned about antibiotics in food fish, especially farmed fish (Claret et al., 2014). Consumer beliefs about the residues of antibiotics in farmed fish impact their views as to the relative wholesomeness and safety of farmed fish vs. wild fish. These unsupported perceptions are an impediment to the growth and acceptance of the aquaculture industry (Verbeke et al., 2007) and also limit consumers' willingness to consume seafood and reap the associated health benefits. As of 1 January 2017, all medically important antibiotics, including those previously approved as over-the-counter products for terrestrial and aquatic livestock, are available only via veterinary prescription or feed directive (FDA, 2017). It is unclear if placing all antibiotics under veterinary oversight will alter usage patterns or change consumer opinions regarding the use of antibiotics in food-producing animals, but it is clear that concerns regarding the use (e.g., volume of drug used, whether regulation and oversight are sufficiently protective of human, animal, and environmental health) or potential misuse of antibiotics (e.g., for unapproved nontherapeutic purposes) in U.S. aquaculture are misplaced and grossly exaggerated in the public sphere. Our results indicate that oxytetracycline does not promote growth or efficiency in farmed fish. It is possible, but perhaps unlikely that other antibiotics could affect fish performance differently. For any putative nontherapeutic effect to incentivize misuse of antibiotics for growth promotion in aquaculture, it would have to outweigh the disincentives related to the additional cost of medicated feed and the risk and difficulty associated with securing drug product for illegal, nontherapeutic use.

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