Effect of feeding during off-flavour depuration on geosmin excretion by Nile tilapia (*Oreochromis niloticus*)

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A B S T R A C T

The effect of feeding during off-flavour depuration on the elimination of geosmin from muscle tissue (fillet) and ovaries as a model for caviar was assessed in Nile tilapia (*Oreochromis niloticus*) (mean ± SD weight of 185 ± 15.0 g). The experiment had a 2 × 4 factorial design with feeding level (starved or fed) and depuration time (24, 48, 72 and 96 h) as factors with duplicates for each of the 8 treatment combinations. Fish were normally loaded with geosmin prior to the experiment.

During off-flavour depuration geosmin levels in fillet and ovary declined over time in both fed and starved tilapia. In fed tilapia geosmin declined faster from the ovaries compared to starved fish ($p = 0.018$). The same trend of a faster decline was observed for the muscle tissue (fillets) of fed tilapia, though only numerically ($p = 0.11$). Because faster geosmin elimination paralleled with high blood lipids, we do not rule out that blood lipids are involved in geosmin transport via the circulatory system and that low blood lipid levels are limiting geosmin elimination in starved fish. No difference in geosmin elimination rate was detected between ovary and muscle tissue in Nile tilapia. Off-flavour depuration time is strongly reduced when farmers adopt a practice of feeding Nile tilapia during off-flavour depuration.

1. Introduction

Geosmin and 2-methylisoborneol (2-MIB) are secondary metabolites produced by a wide range of microbiota common to land-based aquaculture systems. Upon their release to the water these chemicals are quickly bioconcentrated by fish due to their lipophilicity (geosmin log Kow: 3.57 and 2-MIB log Kow: 3.31, Howgate, 2004). The presence of these chemicals in fish tissues causes an earthy or musty off-flavour, which is considered a quality defect by human consumers. Bioconcentration of geosmin and 2-MIB is dynamic and reversible; the chemicals can freely diffuse in and out of the fish via the gills depending on the current fugacity gradient between water and fish (Howgate, 2004). Fish farmers utilize this mechanism to depurate off-flavours from their fish crops by placing them in water free of geosmin and 2-MIB just before harvest. Fish are depurated until excretion has resulted in a reduction of the geosmin and 2-MIB concentrations in the fish to levels below their sensory detection limits. This process however is not always sufficiently effective to prevent market entrance of off-flavoured fish. The general objective of this study is therefore to improve our understanding of the off-flavour depuration process.

Off-flavour depuration generally lasts a few days up to a week, depending mainly on the initial geosmin or 2-MIB levels in the fish and the rates of excretion. Excretion rates depend among other factors on fish size, lipid content and temperature (Howgate, 2004). During off-flavour depuration fish are generally not fed to allow fish to empty their intestinal tracts prior to slaughter and to reduce the water flow over the depuration system required to maintain good water quality.

Geosmin elimination from the fish's body by excretion to the water via the gills requires its transport from peripheral tissues to the gills via the circulatory system. Fasting fish during off-flavour depuration may negatively affect both geosmin transport and excretion through effects on blood lipids and gill ventilation. In blood organic xenobiotic compounds partition between water (plasma) and plasma lipid fractions (Jandacek and Tso, 2001) and hydrophobic xenobiotic compounds with log octanol/water partition coefficients (logKow) larger than three are bound to lipoproteins (Spindler-Vomachka et al., 1984) and specific plasma transporter proteins (Schmieder and Henry, 1988). In rainbow trout, only a minor fraction of moderately lipophilic compounds is

dissolved in the plasma (Schmieder and Henry, 1988). For geosmin this has not been studied, but given its lipophilic nature (logKow of 3.57) it is highly likely that the plasma lipid fraction, including lipoproteins is involved in geosmin transport via the circulatory system. Since starvation affects blood lipid content and composition (Sheridan, 1988; Figueiredo-Silva et al., 2013) an effect of starvation on geosmin transport and subsequent elimination seems likely. Feeding increases gill ventilation to compensate for an increased oxygen demand due to feed induced thermogenesis. Since gill ventilation rate is an important promotor for the rate at which geosmin is excreted via the gills (Howgate, 2004), feeding fish may also promote geosmin exchange over the gills. Given these potential effects of feeding on blood lipids and gill ventilation, we hypothesized that feeding of fish promotes the elimination of geosmin from fish to water during off-flavour depuration. Except for anecdotal accounts of more successful off-flavour depuration in fed fish, there are to the best of our knowledge no scientific records on the effect of starvation or feeding on geosmin elimination from fish. The first objective of the current study was therefore to establish the effects of feeding versus starvation on geosmin excretion by fish.

Normally off-flavour issues relate to the geosmin and 2-MIB levels in fish fillets, but off-flavour may also occur in sturgeon caviar produced in farms. Off-flavours in high-end seafood products such as caviar are obviously not accepted by consumers. In case of caviar the ovary is the organ of main interest. Uptake and elimination of lipophilic chemicals in biota varies among tissues and organs due to differences in perfusion, lipid content and lipid composition (Streit, 1988). We thus hypothesized that off-flavour chemicals are eliminated from the lean muscle tissues (fillet) faster than from ovaries. The second objective of this study was therefore to establish the difference in geosmin elimination rate from fish fillets and ovaries.

As sturgeons with developed ovaries are too large and valuable for use in an experimental setting, we used female tilapia with ovaries as model species. Female tilapia already develop ovaries at small body sizes of 100-200 g. To assess the elimination rates of geosmin from fillet tissue and ovaries of fed and starved tilapia, off-flavoured female tilapia were sampled over time during depuration.

2. Materials and methods

2.1. Ethics statement and origin of the experimental animals

The treatment of the fish was in accordance with Dutch law concerning animal welfare, as approved by the ethical committee for animal experimentation of Wageningen UR Livestock Research (protocol 2013163b). Nile tilapia were progenies of the brood stocks kept at Wageningen University and Research, The Netherlands. Nile tilapia fry was transferred to the research facilities of the institute Wageningen Marine Research, Yerseke, the Netherlands at a mean weight of 5 g.

2.2. Pre-experimental loading of fish with geosmin

Nile tilapia were raised from 5 g to approximately 180 g in a recirculating aquaculture system (RAS) prior to their use in the current experiment over a period of 120 days. In RAS microbial production of geosmin is common (Azaria and Van Rijn, 2018). To create off-flavoured Nile tilapia for the current study, we raised the fish in a RAS system of which we knew that it was contaminated with microbial geosmin. The fish consequently accumulated geosmin during the time spent in this RAS, rendering them off-flavoured at the start of the experiment. During the geosmin enrichment period, fish were fed at a level of 20 g/kg$^{0.8}$/d. The day before their transfer to the experimental systems fish were not fed.

2.3. Experimental design and procedures

The experiment was set up as a 2 × 4 factorial design with two feeding levels (F) and four depuration times (T) as factors. The feeding level treatments (F) were starvation (no feeding) and feeding. Depuration times (T) were 24, 48, 72 and 96 h. The treatments were randomly assigned to 16 experimental units (tanks), with two tanks for each of the eight different combinations of F × T to obtain replication.

At the start of the experiment (T = 0) the fish (n = 120) were randomly split into 20 groups of five fish and weighed per group. Overall mean ± SD body weight was 184.8 ± 15.0 g. Four randomly assigned groups served to determine the initial geosmin content of fish. Each of the 16 other groups was randomly assigned to one of the 16, 180 L polyester tanks. These 16 tanks were randomly assigned to one of the eight treatments.

Experimental tanks were refreshed with local tap water at a measured mean ± SD flow rate of 1263 ± 75 L/d. Water was pumped from a temperature controlled water reservoir to the fish tanks by peristaltic pumps (Watson Marlow 505, Rotterdam, The Netherlands). The water reservoir was continuously supplied with new local tap water. The effluent of the experimental tanks was discharged. The entire experimental facility was set up at 20 °C in a temperature controlled room. Oxygen was supplied to each tank via a minimal flow of pure oxygen instead of air to prevent volatizing of excreted, waterborne geosmin (overall mean ± SD oxygen saturation was 86 ± 15%). Each tank was covered by a 6 mm glass sheet to prevent fish from jumping out of the tanks. The glass cover sheets were equipped with a circular hatch (diameter 150 mm) to allow for introduction of fish. The hatches were covered by glass sheets (200 × 200 mm) during the experiment, leaving a minimal opening for passage of aeration tubing. Water quality parameters were monitored daily in each individual tank up to the moment fish were sampled and the tank removed from the experiment. Mean ± SD temperature was 20.2 ± 0.12 °C and pH ranged between 7.5 and 7.7. The mean ± SD water oxygen saturation was 86.9 ± 15.3% in tanks with starved fish and 81.3 ± 6.7% in tanks with fed fish.

Fish were fed at a rate of 1.1%/d. Feed (Skretting ME-2 Meerval start, 49% protein, 11% crude lipids) was given daily in two equally sized portions (5 g/portion) at 9:00 am and 5:00 pm. To confirm feed intake the presence or absence of uneaten feed pellets was recorded 30 min after each bidaily feeding event. Out of the total of 40 feeding events, left-over feed was observed in 33 cases, indicating that fish were slightly over-fed.

2.4. Sampling

During the experiment fish samples were collected at t = 0, 24, 48, 72 and 96 h, with two tanks and five fish per tank for each treatment. Individual fish weight was measured at stocking (Mettler PM40). For sampling fish were rapidly netted and anaesthetised in 0.1% (v/v) 2-phenoxyethanol (Sigma, St. Louis, USA). Blood samples were taken from each of the five fish per tank by puncture of the caudal vessel with a syringe fitted with a 25-gauge needle. Heparin was used as anticoagulant. The five blood samples per tank were pooled and stored in 5 mL cryo tubes (Greiner bio-one, Germany) at −80 °C until analysis. After blood sampling fish were killed by a blow to the head. Ovaries were dissected, pooled per tank (n = 5) and stored at −80 °C in glass containers until analysis. Fish were then filleted. Fillets were de-skinned, pooled per tank (n = 5), homogenized and stored at −20 °C until analysis. All fish tissue samples were kept on ice during sample collection.

2.5. Geosmin and lipid analysis

Fish fillet and ovary samples were thawed overnight at 4 °C. From each fish fillet sample a subsample of approximately 1 g was taken. To each (sub) sample 100 µl of internal standard solution (D5-geosmine in water, 1 µg/ml, Sigma Aldrich) was added. Samples were extracted by accelerated solvent extraction (ASE, Dionex, Amsterdam, the

Netherlands) at 40 °C using a 15:85 (*v*/v) penthane-dichlorinemethane mixture at 40 °C. After extraction, 1 mL of hexane was added to the extract. Extracts were concentrated to 1 mL by gently evaporating the penthane-dichlorinemethane mixture (Rotavap, Heidolph) and stored in 2 mL amber coloured glass vials at −20 °C until geosmin measurement.

To each water sample of 250 mL 100 μl of internal standard solution (D5-geosmine in water, 1 μg/mL) was added. Water samples were led over an extraction cartridge (Sep-Pak® Vac 6 cc (1 g) Certified tC18) and then eluted with 5 mL diethylether. Water was removed from the collected diethylether by addition of sodiumsulfate. Diethylether samples were concentrated to 1 mL under a gentle nitrogen gas flow and stored in a amber coloured glass vial at −20 °C until geosmin measurement.

For geosmin concentration measurement 1 μL of sample was injected on a Shimadzu GCMS2010 (GC) coupled to a GCMS-QP2010 Ultra (MS) detector (Shimadzu, 's Hertogenbosch, the Netherlands). Analysis was performed in GCxGC mode using a Zoex ZX2 modulator (Shimadzu, 's Hertogenbosch, the Netherlands) with a modulation of 6 s. 1st dimension column was a 30 m × 0.25 mm i.d. HT8 with a film thickness of 0.25 μm. The second dimension was a 2.3 m × 0.25 mm i.d. BPX-50 column with a film thickness of 0.15 μm. Pressure was set at 124.7 kPa. Injection port, interface and source temperatures were set at 225, 290 and 200 °C respectively. The oven temperature was programmed as follows: 60 °C holding for 2 min, then at 15.71 °C/min to 170 °C, then at 5 °C/min to 200 °C. Detection was carried out using electron impact (EI) mode in single ion monitoring (SIM) mode. Quantification was performed using GCMSSolutions software (Shimadzu, 's Hertogenbosch, the Netherlands) with *m/z* 112.1 and 114.1 as quantification ion for Geosmin and D5-Geosmin respectively. Quantification was performed against a calibration curve fitted using 8 points between 1 and 500 ng/g. Geosmin concentration measurements were validated. Lipid content of fillet and ovary samples was determined using a modified version (de Boer, 1988) of the method from Bligh and Dyer (1959).

2.6. Calculations and statistics

In fish tissues geosmin is associated to lipids and variation in lipid content may therefore cause variation in geosmin levels. To remove this variation from our data, geosmin concentrations were normalized for lipid content, i.e. expressed as (ng/g lipid). This was done by dividing the measured geosmin concentrations by the lipid contents of the samples. Outlier analysis detected two outliers for the geosmin level in the fillet of fed fish, one at T_{24} and one at T_{48}. The measured geosmin fillet concentrations were far above the mean geosmin concentration at T_0. The deviation from T_0 was more than three times the standard error of the geosmin concentration at T_0. These two data points were omitted in the data analysis. All statistical procedures were performed in SAS 9.4.

Under the assumption that geosmin does not accumulate in the water because of the continuous supply of geosmin-free water to the tanks, the decline of the geosmin concentration in the lipid fractions of the fillet and the ovary over time $C_{F(t)}$ (ng/g lipid) can be described as exponential decay (Howgate, 2004) (eq. 1):

$$C_{F(t)} = C_{F(t=0)} \, e^{(-k_2 t)} \tag{1}$$

where $C_{F(t=(t=0)}$ is the initial chemical concentration in the fish and k_2 the rate constant (1/h) for the elimination to the water. This equation can be rewritten into a straight line linear regression model (eq. 2):

$$Ln(C_{F(t)}) = Ln(C_{F(t=0)}) - k_2 . t \tag{2}$$

The decline of the geosmin concentration over time was predicted by fitting eq. 2 to the measured geosmin concentrations by non-linear regression analysis. In total four models were fitted, one for each

duplicated combination of feeding treatment (fed or starved) and tissue type (fillet or ovary). This yielded four estimates for slopes and two for intercepts (treatments shared their T_0 samples). The decline of the geosmin concentration over time was tested for treatment effects, i.e. differences in the estimated slopes, using a sum of square reduction test, for both the fillet and the ovary. The significance of the decline of the geosmin concentration over time was assessed for each combination of treatment and tissue by considering the 95% confidence intervals for the estimates of the slopes: the null-hypothesis of no change over time was rejected in case the 95% confidence intervals did not contain zero. To judge the quality of the regression fit, pseudo R^2 values were calculated per tissue type combined for the treatments as (eq. 3):

$$Pseudo \ R^2 = 1 - \frac{Sum \ of \ squares \ Residual}{Sum \ of \ squares \ Total} \tag{3}$$

Under the null-hypothesis of equally fast geosmin elimination from both tissues, the lipid normalized geosmin concentration ratio fillet:ovary does not change over time. This ratio was calculated for each combination of feeding treatment (F) and depuration time (T). The change over time in the geosmin fillet:overy ratio was assessed by linear regression analysis separately for each feeding treatment. Any differences in geosmin elimination between fillet and ovary would be detected by a slope that is significantly different from zero.

Blood lipid content at T_{24}, T_{48}, T_{72} and T_{96} were analysed for the effect of feeding treatment (starved vs. feeding) and the interaction between treatment and time by two-way ANOVA. Tukey post hoc analysis was done to compare individual means.

3. Results

3.1. Raw data

An overview of the geosmin concentrations in the fillets and ovaries and the blood lipid content observed in the experiment is presented in Table 1.

3.2. Treatment effects on geosmin elimination

Both fed and starved Nile tilapia eliminated geosmin from their muscle tissues (fillet) and ovaries in a time dependent manner: all estimated slopes are negative and significantly different from zero (Table 2). The fed fish showed faster elimination of geosmin from their ovaries than the starved fish ($p = 0.018$, Fig. 1). No treatment effect on geosmin elimination from the fillet was detected ($p = 0.11$, Fig. 1), but numerically also in the fillet feeding increased the elimination rate.

3.3. Tissue effects on geosmin elimination

No tissue effects on geosmin elimination were detected as the lipid normalized geosmin concentration ratio fillet:ovary did not change over time for both the fed ($p = 0.71$) and the starved Nile tilapia ($p = 0.33$) (Fig. 2).

3.4. Treatment effects on blood lipid content

Blood lipid content per feeding treatment and per sampling time is given in Table 3. Blood lipid content averaged over time points was higher in fed fish than in starved fish (two-way ANOVA, $P_{Treatment} = 0.001$). However, the effect of feeding treatment on blood lipid content altered with time, being indicated by the interaction effect between feeding treatment and time ($P_{Treatment*Time} = 0.008$). Comparison of means per time point and treatment by post hoc analysis revealed that blood lipid content changed during the course of the depuration experiment in the fed fish but not in the starved fish (Table 3). In the fed fish blood lipid increased with time during depuration and was highest at T_{72} and T_{96} (Table 3).

Table 1

Geosmin concentrations in fillets and ovaries an the lipid content of fillets, ovaries and blood samples as observed in the 20 experimental groups. The 20 experimental groups represent the replicated samplings at five time points for two treatments. Each group consists of a pooled sample of five individual fish.a), b)

			Fillet			Ovary			Blood
			Lipid	Geosmin	Geosmin	Lipid	Geosmin	Geosmin	Lipid
Treatment	Replicate	Time	(%, w/w)	(ng/g)	(ng/g lipid)	(%, w/w)	(ng/g)	(ng/g lipid)	(%, w/w)
.	1	0	3.6	5.1	142	21.4	38	178	1.6
.	2	0	3.2	5.0	156	19.4	36	186	1.6
.	3	0	3.8	4.4	116	19.2	36	188	1.4
.	4	0	5.2	4.1	79	21.1	44	209	1.3
Fed	1	24	3.5	2.0	57	19.1	31	162	1.2
Fed	2	24	2.8	11	393[b]	19.5	32	164	1.0
Not Fed	1	24	3.8	4.1	108	20.4	32	157	1.3
Not Fed	2	24	3.5	5.3	151	19.2	28	146	1.5
Fed	1	48	3.2	10	313[b]	18.9	21	111	2.0
Fed	2	48	3.9	2.8	72	20.4	32	157	1.6
Not Fed	1	48	3.2	2.6	81	19.7	27	137	1.5
Not Fed	2	48	3.4	5.3	156	18.9	29	153	1.0
Fed	1	72	3.7	1.9	51	19.4	18	93	2.1
Fed	2	72	2.9	1.8	62	18.8	22	117	2.0
Not Fed	1	72	4.5	2.6	58	19	21	111	1.4
Not Fed	2	72	5.4	5.9	109	19.2	26	135	1.3
Fed	1	96	3.2	1.7	53	19.9	18	90	2.1
Fed	2	96	1.9	< 1.3[a]	34	18.5	15	81	2.2
Not Fed	1	96	3.4	1.3	38	19.5	23	118	1.3
Not Fed	2	96	3.6	2.1	58	21.4	23	107	1.3

[a] value below the analytical detection limit of 1.3 ng/g. Considered in the data analysis as half of the detection limit: 0.65 ng/g.

[b] outliers omitted from the data analysis.

Table 2

Results of the linear regression analysis of the development over time of the natural logarithm transformed geosmin concentrations for the fillets and ovaries of fed and starved Nile tilapia.

Tissue	Model parameter	Estimate	s.e.	95% CI LL	95% CI UL
Fillet	Intercept	4.8155	0.0985	4.6093	5.0217
	Slope k_2 – Starved	−0.0074	0.00217	−0.0119	−0.0028
	Slope k_2 – Fed	−0.0114	0.00217	−0.0159	−0.0069
Ovary	Intercept	5.2402	0.0283	5.1814	5.299
	Slope k_2 - Starved	−0.0057	0.00063	−0.007	−0.0044
	Slope k_2 - Fed	−0.0081	0.00063	−0.0094	−0.0067

4. Discussion

Feeding Nile tilapia during off-flavour depuration resulted in a significant higher elimination rate of geosmin from the ovary. Our data suggest the same effect for the muscle tissue (fillets), but for fillets the feeding treatment gave only a numerical difference. We attribute this to the relatively higher analytical variation in the data caused by the low (initial) geosmin levels in fillets rather than absence of an enhancing effect of feeding during off-flavour depuration on geosmin elimination from the fillets.

Geosmin excretion by fish predominantly takes place via exchange between blood and water in the gills (Howgate, 2004). For depuration of off-flavours this implies the need of geosmin transport from peripheral tissues to the gills via the circulatory system. We hypothesized that geosmin would be eliminated faster from fed fish than starved fish due to higher blood lipid content and gill ventilation in the fed fish. The higher level of blood lipids, assumed to be mainly present as lipoproteins (Sheridan, 1988), could increase the transport capacity of the circulatory system while the higher gill ventilation promotes excretion from blood to water over the gills. As the amount of lipid in the fish' blood depends, among other factors, on the nutritional status of fish (Sheridan, 1988), we could successfully create differences in blood lipid content between the treatments by feeding and not feeding the groups of experimental fish. In the starved groups the blood lipid content remained unchanged during the experiment at levels ranging from 1.25 to

1.40% (w/w). In the fed groups the blood lipid content was higher at T_{48} and T_{72} compared to T_0, which is most likely due to the fact that during the last day of geosmin enrichment (< T_0) fish were not fed.

Our observation that fed fish with higher blood lipid levels eliminate geosmin faster from their ovaries and possibly their fillets suggests that geosmin excretion in starved fish is limited by low blood lipid levels. It should be noted however that our data do not provide direct evidence for binding of geosmin to blood lipids nor causality between blood lipid levels and geosmin transport and excretion. The efficacy of uptake across the gills as well as the transport capacity of blood depend on the capacity of compounds to bind to various plasma proteins (Streit and Siré, 1993; Schmieder and Weber, 1992). Given their high binding capacity to plasma lipoproteins, uptake of lipophilic compounds with logKow > 3 across the gill is considered not to be limited by transport capacity of blood (Schmieder and Weber, 1992). Whether this also applies to the reverse process, i.e. excretion of lipophilic compounds over the gills remains undocumented. In contrast to uptake, excretion rates of moderately lipophilic compounds depend on the total lipid volume in the fish (Gobas and MacKay, 1987) but the contribution of blood lipids to the total lipid volume is small. Any effect of blood lipids on geosmin excretion is therefore more likely related to a functional role in geosmin transport than to a contribution to the total lipid volume. Considering that transport of geosmin from peripheral tissues to the gills requires the exchange of geosmin between tissue and blood lipid compartments, it seems quite plausible that a larger blood lipid compartment leads to faster excretion and that, in contrast to uptake, low blood lipid can limit excretion as our observations suggest. Uptake of lipophilic compounds is limited by its delivery at the gill surface, i.e., the gill ventilation rate (Schmieder and Weber, 1992). Gill ventilation rate is also an important determinant for the rate of excretion across the gills of lipophilic compounds (Gobas and MacKay, 1987) including geosmin according to Howgate (2004). We consequently attributed the positive effect of exercise on geosmin excretion in eel to physiological adaptions, including increased gill ventilation, needed to handle the exercise induced increased oxygen demand (Schram et al., 2016). Feeding also increases oxygen demand in fish (Jobling, 1981). Our observations corroborate with this finding, in the tanks with fed fish
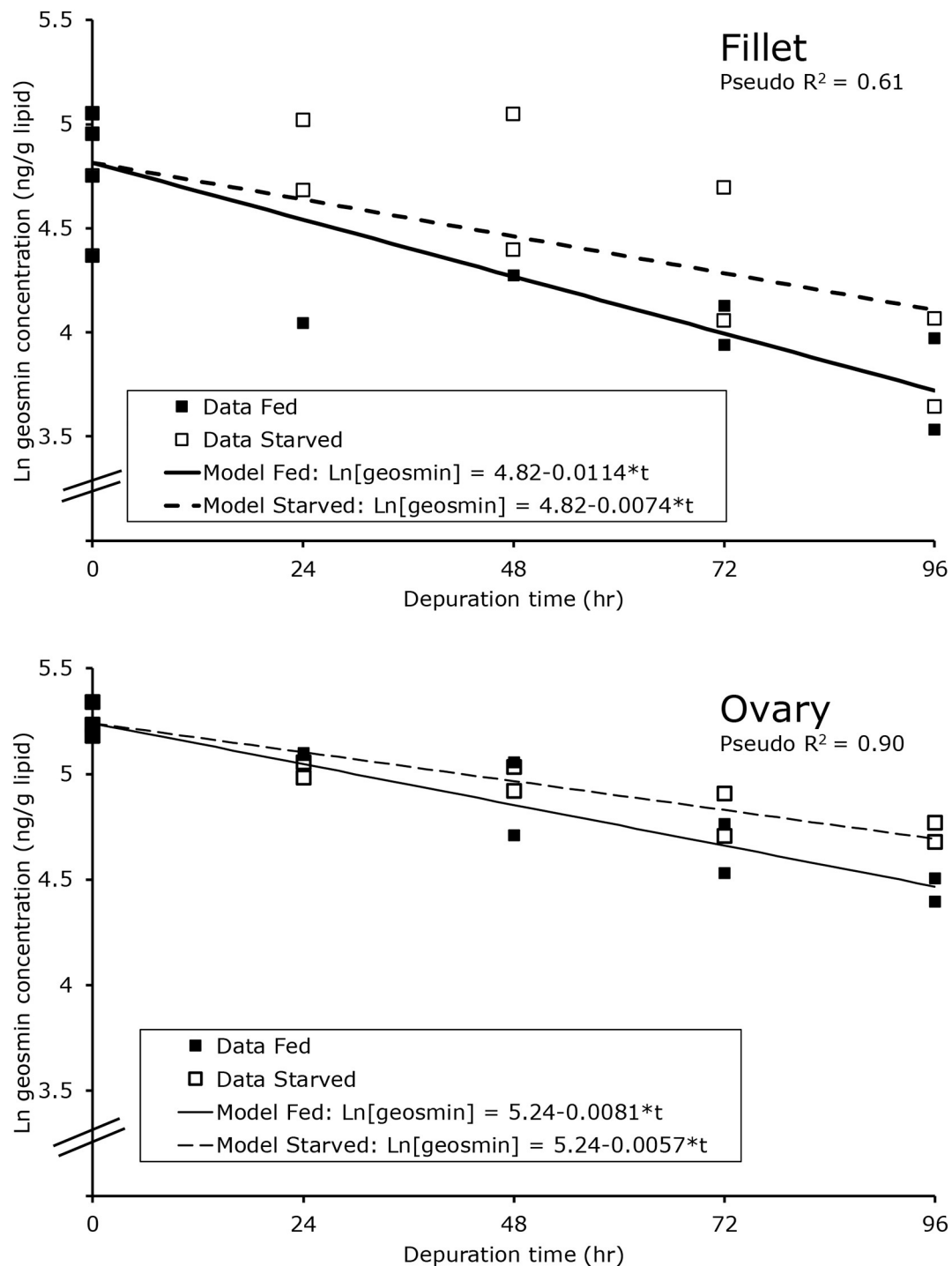
Fig. 1. Decline of natural logarithm transformed geosmin concentrations over time from the fillets (top) and ovaries (bottom) of Nile tilapia that were fed or starved during off-flavour depuration.

mean oxygen saturation levels were slightly lower (81.3% compared to 86.9%). Liver activity and perfusion may also be increased in fed fish. Given that there is some indirect evidence suggesting geosmin biotransformation in the liver of rainbow trout (Schram et al., 2018) and biotransformation rate may be increased with increased metabolism, geosmin elimination through biotransformation may be increased in fed compared to starved fish. We do not rule out that geosmin is partially excreted via the faeces, and this intestinal excretion may have been enhanced in the fed fish due to a higher content of the intestinal tract compared to the starved fish.

Although our data seem to suggest faster elimination from the fillets

(Table 2), no significant tissue effect was detected in this study (Fig. 2). This is surprising considering that the fillets were around a fivefold leaner (lipid content ~3.6% w/w) than the ovaries (lipid content ~19.6% w/w) and the rate at which lipophilic compounds are eliminated from tissues decreases with increasing lipid content (Gobas and MacKay, 1987). Differences in perfusion between muscle tissue and the ovary may also have played a role, where higher perfusion led to faster elimination (Streit, 1988). However, without information on the perfusion of the ovary relative to the fillet it is impossible to evaluate whether or not higher perfusion to some extend counteracted the negative effect of the higher lipid content on the elimination rate of
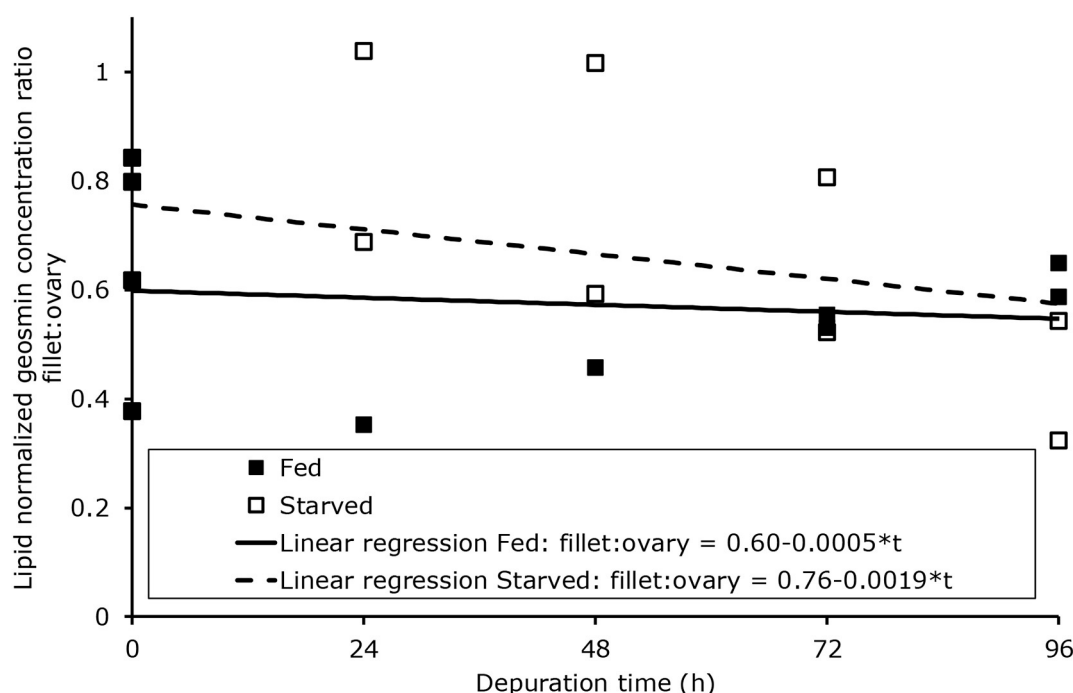
Fig. 2. Lipid normalized geosmin concentration ratio fillet:ovary over time for Nile tilapia that were fed or starved during off-flavour depuration.

Table 3

Mean $\pm$ SD blood lipid content per treatment (Starved or Fed) and sampling time. Mean values marked with different letters in superscript are significantly different ($p < 0.05$, Tukey, post hoc analysis.

Time (h)	Blood lipid content (%)	
	Starved fish	Fed fish
0	1.48 ± 0.15	
24	1.40 ± 0.14[ab]	1.10 ± 0.14[a]
48	1.25 ± 0.35[a]	1.80 ± 0.28[abc]
72	1.35 ± 0.07[ab]	2.05 ± 0.07[bc]
96	1.30 ± 0.00[a]	2.15 ± 0.07[c]

geosmin from the ovary.

The current observation of a faster elimination of geosmin in fed fish has several implications for industrial depuration practises. Fish farmers use the reversibility of geosmin bioconcentration to depurate off flavours from their fish crops by placing them in water free of geosmin just before harvest. The time required for successful depuration is then largely determined by the initial levels of off-flavour compounds and their elimination rates (Howgate, 2004). Higher initial geosmin levels lead to longer depuration times to reach tissues concentrations below sensory detection limits, more so when elimination rates are lower. In general fish are not fed during depuration. Only in case of long-term depuration of large fish such as sturgeons, fish are occasionally fed (Sindilariu, pers. comm.). Because one of the practical reasons for not feeding fish during depuration is to allow the fish to empty their intestinal tract before slaughther, it is probably not possible to feed fish up to the last day of the depuration period. Also changing conditions and fish handling at the start of the depuration period may cause an initial decline in feed acceptance. It thus seems clear that feed intake during the depuration period will be lower than during the preceeding production phase. To what extent this limits the room for enhancing geosmin excretion by feeding the fish remains unclear until the effect of feeding level on geosmin excretion has been clarified.

Another practical reason for not feeding fish during off-flavour depuration is that it would place additional demands on the depuration system to handle the higher waste production of the fish. In case fish are fed, the water in the depuration system has to be renewed more frequently or recycled after internal purification to maintain proper water quality. The first action increases water demand which conflicts with farming fish in recirculating aquaculture systems (RAS) with the objective to reduce water demand. The second action (water recycling) requires biological filters. Since these are potential sources of off-flavour chemicals (e.g. Azaria and Van Rijn, 2018), this may lead to geosmin and 2-MIB exposure in the depuration system and a longer off-flavour depuration time. Clearly the practical implications of feeding fish during off-flavour depuration remain to be elucidated.

To illustrate that the practice of starving fish during off-flavour depuration leads to suboptimal performance of the depuration process we used our estimates for the geosmin elimination rates to calculate the time required to reach an arbitrary sensory detection limit of 25 ng/g lipid for all four combinations of treatment and tissue. This sensory detection limit corresponds to 0.9 ng/g in the fillet and 4.9 ng/g in the ovary based on the respective average lipid contents. We thus take into account that higher lipid levels concur with higher sensory detection limits (Howgate, 2004). To reach the sensory detection limit in the fillets the required depuration times are 140 h for the fed fish and 216 h for the starved fish. This seemingly large effect of feeding the fish during off-flavour depuration is remarkable considering the absence of significant treatment effects. Although it should be treated with caution because we extrapolated the effects in time far beyond the duration of our experiment, it does illustrate that relatively small differences in elimination rates, which may be difficult to detect experimentally, can have strong impacts on the longer term. For the ovaries the required depuration times to reach a geosmin concentration of 25 ng/g lipid are 251 h for the fed fish and 354 h for the starved fish. Again the starved fish need more time to eliminate geosmin and given the significant feeding treatment effect this is not surprising.

More remarkable are the differences between the fillet and the ovary. Irrespective of the feeding treatment, the fish needed more time to eliminate geosmin from their ovaries compared to the fillets. Since the geosmin elimination rates did not significantly differ between fillets and ovaries this difference in required depuration time can be largely attributed to the higher initial geosmin levels in the ovaries. Clearly Nile tilapia bioconcentrates more geosmin in its ovaries than in its

fillets under a given geosmin exposure. Normalizing geosmin levels for lipid content reduced the average ratio fillet:ovary for the initial geosmin level from 0.12:1 to 0.6:1. This indicates that the higher lipid content largely but not entirely explains the higher geosmin level in the ovary. In line with our previous findings (Schram et al., 2018) it seems that geosmin distribution within the fish is not exclusively governed by the lipid content of tissues.

The finding that fish bio-concentrate geosmin to higher levels in their ovaries and consequently need more time for depuration compared to muscle tissues is relevant for off-flavour depuration in the aquaculture production of sturgeons for caviar and meat. Given the contrast in lipid content of sturgeon ovaries (~28–37%, Ovissipour et al., 2015) and meat (~6–10%, Jankowska et al., 2002; Vaccaro et al., 2005) it is likely that sturgeons will accumulate more geosmin in their ovaries than in their muscle tissue. In that case the time required for successful off-flavour depuration is probably determined by the ovary and not the fillet. This is confirmed by practical observations at commercial sturgeon farms (Bonpunt, pers. comm.). It also means that using the sensory quality of fillets as indicator for the sensory quality of the ovary may result in the harvest of still off-flavoured caviar. It should be noted that the use of female tilapia as a model for sturgeon was a pragmatic choice, i.e. readily available small fish with developed ovaries, and not based on similarities in physiology.

In conclusion, Nile tilapia that are fed during off-flavour depuration eliminate geosmin faster from their ovaries when compared to starved fish. We attribute this mainly to increased gill ventilation induced by an increased oxygen demand. At the same time we do not rule out that low blood lipid levels in starved fish limit geosmin elimination.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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