Aquat. Living Resour., 1997, **10**, 343-350

Voluntary feed intake and discrimination of diets containing a novel fluoroquinolone in self-fed rainbow trout

Thierry Boujard [(1)] and Raphaela Le Gouvello [(2)]

[(1)] *Unité mixte INRA-IFREMER de Nutrition des Poissons, Station d'Hydrobiologie INRA, 64310 Saint-Pée-sur-Nivelle, France.*
E-mail: boujard@st-pee.inra.fr

[(2)] *STERMOR, Kerfahler 56760 Pénestin, France.*

Received April 21, 1997; accepted August 25, 1997.

Boujard T., R. Le Gouvello. *Aquat. Living Resour.*, 1997, **10**, 343-350.

Abstract

The objective of the study was to evaluate the capacity of rainbow trout to discriminate diets containing micro-granulated or powdered novel fluoroquinolone in comparison with a positive (containing sulfamerazine sodium) and a negative control (placebo). In a first trial, groups of trout had access to a self-feeder containing the placebo feed for 10 days, and then to one of the four feeds during another period of 10 days. Fish reduced their voluntary feed intake (VFI) by more than 50 % when they had access to the positive control, and by more than 20 % when they had access to feed containing micro-granulated premix. There was no significant decrease in VFI when the fish had access to feed containing powdered premix, but in this latter case, an increase in feed refusals was observed. In a second trial, the capacity of the trout to discriminate between two self-feeders containing different feeds was tested. During a first period of 10 days, all the feed hoppers were loaded with the placebo feed, to determine the feeder preference relative to any dietary-independent factor. In the following period of 10 days, fish had access simultaneously to the two medicated feeds. During a third period of 10 days, a validation of the results was attempted, using the placebo feed or the positive control in one of the feeders. It was shown that trout were able to discriminate between the two medicated feeds and that micro-granulated premix was better accepted than powdered premix. In addition, an increase in feed refusals was observed when the preferred feeder was loaded with feed containing powdered premix. It is concluded that this tested fluoroquinolone could decrease significantly VFI. The powdered premix was more detectable by the fish than the micro-granulated premix and led to some refusals.

Keywords: *Oncorhyncus mykiss*, rainbow trout, self-feeding, voluntary feed intake, antibiotics.

Consommation volontaire et discrimination d'aliments contenant une nouvelle fluoroquinolone par des truites arc-en-ciel nourries avec des distributeurs à la demande.

Résumé

L'objectif de ce travail est d'évaluer la capacité de truites arc-en-ciel à distinguer deux aliments contenant une nouvelle fluoroquinolone incorporée sous forme de micro-granules ou de poudre en comparaison avec un aliment témoin (contenant de la sulfamérazine) ou un contrôle (aliment placebo). Dans une première expérimentation, des groupes de truites ont accès à un distributeur à la demande contenant l'aliment placebo durant 10 jours, puis à l'un des quatre aliments durant une période successive de 10 jours. Les poissons réduisent leur consommation volontaire d'aliments de plus de 50 % lorsqu'ils ont accès à l'aliment témoin, et par plus de 20 % lorsqu'ils ont accès à l'aliment contenant l'antibiotique préparé sous forme de micro-granulés. Il n'y a pas de diminution significative de la demande d'aliments lorsque celui-ci contient l'antibiotique en poudre, mais dans ce cas la quantité d'aliments non consommés augmente. Dans une seconde expérimentation, la capacité des truites à différencier deux distributeurs d'aliments contenant les deux différentes formulations d'aliments médicamenteux a été testée. Durant une première phase de 10 jours, tous les distributeurs contenaient de l'aliment placebo, afin de déterminer la préférence éventuelle des distributeurs en relation avec des paramètres indépendants de l'aliment lui-même. Puis durant une seconde période de 10 jours, les poissons ont accès simultanément aux deux aliments médicamenteux. Enfin, durant une ultime période de 10 jours, une validation des résultats est tentée, en utilisant l'aliment placebo ou l'aliment témoin dans l'un des deux distributeurs. On montre ainsi que les truites sont capables de distinguer les deux aliments médicamenteux, et que l'incorporation de l'antibiotique sous forme de micro-granules est mieux acceptée par ces dernières que l'incorporation sous forme de poudre. De plus, une augmentation des aliments refusés est observée lorsque le distributeur d'aliments le plus utilisé par le groupe de truites est utilisé pour l'aliment contenant l'antibiotique incorporé sous forme de poudre. En conclusion, la fluoroquinolone diminue significativement la consommation volontaire d'aliments, et la préparation en poudre est plus facilement détectable par le poisson que la préparation sous forme de micro-granules.

Mots-clés: Truite arc-en-ciel, distributeurs à la demande, appétit, acceptabilité, antibiotiques.

INTRODUCTION

Some drugs have appeared to be very unpalatable to fish at recommended concentrations in the feed. Schreck and Moffitt (1987) found that fish offered pellets by hand containing erythromycin thiocyanate even at a concentration of 0.6 %, took significantly longer time to strike the pellets, ejected individual pellets more often and took a longer time to consume the pellets than fish offered pellets containing no antibiotic. Moreover, the percentage of uneaten pellets reached 25, 20 and 10 % with diets containing 1.2, 1 and 0.6 % of erythromycin thiocyanate, respectively.

Poe and Wilson (1989) and Robinson *et al.* (1990) demonstrated that medicated feeds containing Romet-30®, one of the two antibiotics most used in the US catfish industry, containing sulfadimethoxine and ormetoprim, was not well consumed at the recommended concentration of 3.3 % in feed and led to a dramatic reduction of daily feed intake by 50 %. In the Robinson *et al.* (1990) experiment, palatability of feed was still poor when the concentration of Romet-30® was half the recommended concentration. Hustvedt *et al.* (1991), also demonstrated that 1 % of oxolinic acid or oxytetracycline tended to reduce feed intake in rainbow trout by 17 and 61 %, respectively.

Bowser *et al.* (1990), demonstrated that quinolones like oxolinic acid and enrofloxacin could be successfully used for the treatment of *Aeromonas salmonicida* at 10 mg. kg^{-1}. d^{-1} for 10 days in a hybrid brook trout. The treatment was not successful when the same drugs were administered at 5 mg. kg^{-1}. d^{-1} for five days to Atlantic salmon. The authors did not collect any information concerning the palatability of the medicated feed, so the lack of efficacy in the salmon trial may have been due to a lack of acceptance of the medicated ration. Indeed, since diseased fish may not feed well, poor palatability of medicated feeds may lead to a dramatic decrease in the dose really administered to fish, and therefore induce a lower efficiency of the treatment.

It is obvious that real intake of medicated feed is linked to its acceptance by the fish. In this study, our major concern was to evaluate the possibility of using self-feeders to measure feed acceptance and discrimination of medicated feed in comparison with other feeds. For this purpose, we used a novel fluoroquinolone, known to be effective for the treatment of various rainbow trout and salmon diseases. We used two premixes containing the same amount of the same active ingredient under a different formulation. In a first trial, the effect of incorporating a novel fluoroquinolone in the diet of rainbow trout on their voluntary feed intake was compared with a placebo feed and a positive control. A second trial was performed to evaluate the capacity of rainbow trout to discriminate between the two premixes presented simultaneously by means of self-feeders (choice situation).

MATERIALS AND METHODS

Feed preparation

Four batches of feed of the same composition were prepared in the INRA facilities with a small laboratory press in the following order:

feed *a* : placebo feed (Table 1);

feed *b* : placebo feed + 10 g.kg^{-1} of a micro-granulated premix with 20 % of novel fluoroquinolone;

feed *c* : placebo feed + 10 g.kg^{-1} of a powdered premix with 20 % of novel fluoroquinolone;

feed *d* : placebo feed + 10 g.kg^{-1} of sulfamerazine sodium (Sigma chem.).

Between each feed process, the press was cleaned with 5 kg of corn starch. An aliquot of the feed *a* was analysed following the usual procedures: dry matter (110 °C for 24 h), crude protein (Kjeldahl, total nitrogen × 6.25) after acid digestion, lipid extraction by petroleum ether in a Soxhlet apparatus after acid hydrolysis, energy using a Gallenkamp adiabatic calorimeter. Digestibility trial was previously (Boujard and Médale, 1994) performed with fish fed twice a day the experimental diet containing 1 % of chromic oxide as an inert tracer. Faeces were collected over a 15-day

Table 1. – Ingredients, chemical composition and apparent digestibility coefficients of the experimental diet.

Ingredients (%)	
Fish meal (Descal)	40
Soluble concentrate of fish protein (Sopropêche)	5
Fish oil (Sopropêche)	12
Gelatinized corn starch (Roquette)	28
Corn gluten	10
Vitamin mix (INRA normes EIFAC, 1971)	2
Mineral mix (INRA recommendations Luquet, 1971)	1
Na-alginate	2
Chemical composition	
Dry matter (dm) (%)	88.3
Proteins (N × 6.25) (% dm)	37.9
Lipids (% dm)	18.5
Gross energy ($kJ.g^{-1}$ dm)	22.4
Ash (% dm)	8.8
Apparent digestibility coefficients	
Proteins (%)	89.6
Energy (%)	88.2
Digestible protein/digestible energy	17.2

period using a continuous automatic faeces collector (Choubert *et al.*, 1982). The digestibility of the dietary nutrients were calculated as outlined by Kim and Kaushik (1992). Information concerning the ingredients, the chemical composition and the apparent digestibility coefficients of the experimental diets are summarised in Table 1.

The final concentration of fluoroquinolone was checked in samples of each feed by HPLC, and was found to be < 0.0003 % in feed *a* (detection limit for fluoroquinolone assay in feed), 0.20 % in feed *b*, 0.20 % in feed *c*, respectively. The amount of active ingredient in feed *b* and *c* corresponds to what is recommended by the manufacturer.

Fish adaptation and description of the experimental unit

All the fish (rainbow trout, *Oncorhynchus mykiss*) came from the same parental stock, and were produced in the INRA facilities of Lees-Athas during the Winter of 94. On Feb 28, 18 groups of 30 trout were randomly constituted and distributed among 18 fibreglass tanks of 100 l each. The tanks were part of two units of recirculating water of 12 tanks each. A 1.5 $l.min^{-1}$ input of water from the city was allowed permanently in each of the recirculating units. Water flow of each tank was 3 $l.min^{-1}$. Water temperature was electronically monitored and maintained at 16 ± 0.2 °C.

Fish were fed by means of electronic self-feeders. This feeding system was designed in such a way that each time a fish activated a rod, a predetermined amount of food was delivered (between 0.3 and 0.5 g, *i.e.* 4-8 pellets). It consisted of 3 parts, the detector, the feed hopper, and the interface between the detector and the feed hopper (Boujard *et al.*, 1992). The detector, a magnetic proximity switch (type IFR-12-24-26 NPN, Baumer electric Inc., Switzerland), was positioned at the apex of a rod, that pivoted freely around its axis and that was positioned 1 cm above the water surface in order to prevent any unintentional triggering by the fish. The detector closes a logical 5 V direct current circuit when the rod moves, without any contact between the rod and the switch. The detectors and the feed hoppers (type D-50, RENNA Inc., France) were interfaced with a simple electronic 6 channels device used to transform the low DC pulse into a 3 second' 12 V current through a relay (Boujard *et al.*, 1992).

Demands were rewarded only during two phases of 2.5 h per day each in order to oblige fish to feed twice daily as in fish farming conditions. Fish were conditioned to restrict their feeding activity to the restricted feeding time phases, by means of an additional 40 W light that was switched on only during these feeding phases. Feeding time phases were 06:00-08:30h and 18:00-20:30 h, and the light/dark cycle was 15.5h/8.5h.

Trials 1 and 2 began simultaneously on March 23 (D0). For each tank, the fish were counted and the total biomass was measured on D0 (N_0 and W_{D0}), D21 (N_{21} and W_{D21}) in trial 1 and D31 (N_{31} and W_{D31}) in trial 2. Each day of the trials, the remaining feed contained in each feed hopper was weighed, and some feed was added if necessary. Feed wastes were evaluated immediately after each meal by counting the amount of uneaten pellets in the sediment traps that equipped the water outlet of each tank and multiplying by their mean dry weight (0.07 g).

Twelve tanks were used for trial 1. From D1 to D10, all tanks were fed by means of self-feeders containing feed *a*. From D11 to D20, feed *a* was replaced by feeds *b*, *c* or *d* in 9 tanks (3 tanks per feed). Feeds *b*, *c* and *d* were assigned randomly to the different tanks.

Six tanks were used for trial 2. In order to evaluate the capacity of the fish to discriminate between feeds *b* and *c*, these tanks were equipped with two self-feeders. From D1 to D10, all the feeders contained feed *a*. The feeder preference was determined by daily weighing of each feeder content. The preferred feeder was assumed to be the one which delivered on average more than 50 % of the feeds during the first 10 days. In the remaining days of this trial, the experimental protocol was as follows :

• From D11 to D20, the objective was to detect if the location of the two medicated feeds into the two feeders could modify their use, indicating feed preference. For this purpose, in 3 tanks the preferred feeder was filled with feed *b*, and feed *c* was placed into the other feeder. In the 3 other tanks, the preferred feeder was filled with feed *c*, and feed *b* was placed into the other feeder.

• From D21 to D30, the objective was to compare the behaviour of the fish having access to the positive or to the negative control. For this purpose, when a shift in the feeder preference was observed, the previously preferred feeder was filled with feed *a*, and the other feeder was filled with the same feed than during the previous 10 day period (feed *b* or *c*) ; but when no shift in the feeder preference was observed, the preferred feeder was filled with feed *d* and the other feeder was filled with feed *a* in order to force the fish to change their feeder preference.

The following parameters were used to analyse the results:

• Feed demand (FD) = demanded feed / days;

• Feed refusal (FR) = feed waste / days, expressed in % of FD;

• Voluntary feed intake (VFI) = (demanded feed - feed waste) / days.

• Specific growth rate (SGR) = 100 (ln(final weight) - ln (initial weight)) / days;

• Feed gain ratio (FGR) = (total demanded feed - total feed waste) / (final weight - initial weight).

• Daily amount of fluoroquinolone ingested = 0.2 % of VFI, expressed in $mg.kg^{-1}.d^{-1}$.

FD, VFI, FGR and SGR were analysed for normality of variance, and by ANOVA with the SAS package (proc GLM, SAS Inst. Inc., NC, USA). Arcsine transformations of percentage data were performed to achieve homogeneity of variance. When F values indicated significance, individual means were compared using Duncan multiple range test ($p < 0.05$).

RESULTS

First trial

Overall mortality was 1.4 %, and the cause of death was not elucidated. The daily feed demand, FD (Fig. 1*a*) is comprised between 1.5 and 2.5 % of the body weight during the whole duration of the trial when fish were fed with feed *a*. The change to medicated feeds *b*, *c* and *d* provoked an immediate drop in FD. This drop was paralleled by an increase in feed refusals in tanks fed with feed *c* only (Fig. 1*b*). It should also be noted that the amount of feed *c* refusals is decreasing on D18-D20, simultaneously with a decrease in FD.

SGR was significantly affected by the type of feed used during the second part of the trial, in relation to a significant decrease in feed intake when fish were fed with the medicated feeds. This decrease in feed intake was of more than 50 % with feed *d*, and approximately 30 % with feed *b* (Table 2).

Second trial

Overall mortality was 1.8 %, and the cause of death was not elucidated. Growth and feed conversion (Table 3) were not significantly affected by the feeding protocols (ANOVA, $p < 0.05$), and not different to the results obtained in trial 1 for fish fed with feed *a*. The VFI was also similar with the VFI observed in trial 1 during the first ten days, with values close to 2 %. Changes in feed intake between the period D1-D10 and D11-D20 were not significant.

When studying the evolution of the daily demands in the two different self-feeders located in each tank, it was seen that one of the two feeders was always preferred. Indeed, during the first ten days of the experiment, when all the feeders were loaded with the same feed, a preferred feeder was identified, with approximately 60-70 % of the demands, in each tank (Table 4).

During the next 10 days of the experiment, when the preferred feeder was loaded with feed *b* and the other feeder with feed *c*, no shift in preference could be detected (Table 4, Fig. 2), while in the other triplicates, where the preferred feeder was loaded with feed *c* and the other feeder was loaded with feed *b*, a significant shift of approximately 23 %, was observed for the total period D11-D20.

In fact, it can be seen that this decrease in the use of the preferred feeder appeared only after 7 days on average (Fig. 2). It is interesting to note that during the last part of this trial, the preferred feeder, filled up with feed *a* and previously filled up with feed *c*, was again used significantly more than the other after 3 days. When the preferred feeder was filled up with *d* (previously filled up with feed *b*), the observed decrease in use was not significant.

DISCUSSION

Anthouard and Wolf (1988), presented several examples of events that affect temporarily the use of self-feeders. Among other, they showed that *Oreochromis mossambicus* decreased significantly its FD after a temporary stoppage of water oxygenation. The European catfish (*Silurus glanis*) also showed decreasing FD that paralleled temperature decrease. During the course of an experiment that necessitate periodic weighing of fish, Kentouri *et al.* (1994), showed that FD was depressed each days of weighing. These works demonstrate that self-feeders are well adapted for studying the influence of external factors on feeding activity in fish. The influence of dietary factors such as DE content on feed demands of self-fed Atlantic salmon and rainbow trout has also been demonstrated (Boujard and Médale, 1994; Paspatis and Boujard, 1996).

The decrease in FD and VFI observed when using the positive control was therefore a predictable result and validates the use of self-feeders for studying the palatability of different feeds. Feed *b* also decreased significantly FD, indicating that the premix used affects palatability of the feed. Feed *c* did not decrease significantly the FD, but an increase in FR and FGR was observed. This suggests that palatability of this feed is also affected by the premix used, but in a different manner than in feed *b*.

The first experiments that demonstrated the ability of fish to discriminate between two triggers was by Adron *et al.* (1973) with rainbow trout. They wondered if some actuation may result from accidental collision

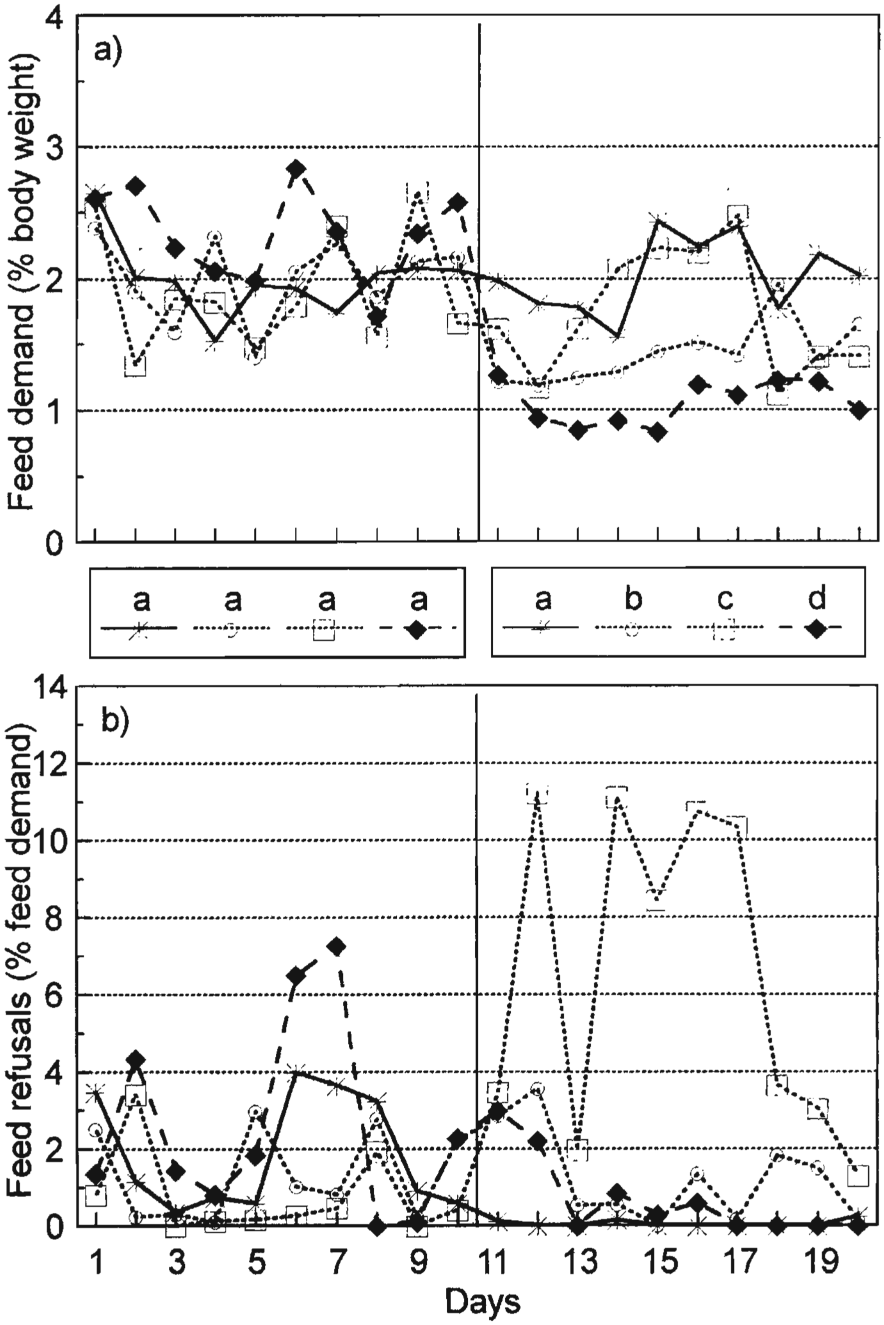

Figure 1. – Evolution of (*a*) the daily mean feed demand, and (*b*) the daily mean refusals for the different treatments during the course of trial 1.

of the body of a fish with the trigger. In order to determine the importance of accidental actuation of their feeders, they presented well trained trout with a choice between two triggers, one connected with a food dispenser but not the other. The ability to discriminate towards the food trigger was obvious within hours. Adron *et al.* (1973), also presented groups of trained fish with a choice between two identical self-feeders that distributed two different diets, the first containing casein and considered relatively bland, and the second containing fish protein, or shrimp extract, or mussel extract. Within 2 to 4 days, a clear and consistent preference for diets containing shrimp and mussel extract was shown, with 60-65 % of the demands for these

Table 2. – Growth and feed ingestion during the first trial. Effect of the use of the medicated feeds *b*, *c* and *d* on feed gain ratio, specific growth rate, voluntary feed intake during the first and the last 10 days, and the decrease in voluntary feed intake between the first and the last ten days. The total amount of active ingredient ingested is also indicated. Data are shown as means ± SD (n = 3).

Feeding protocol					Statistical analysis
D1-D10	*a*	*a*	*a*	*a*	
D11-D20	*a*	*b*	*c*	*d*	
Growth parameters					
Feed gain ratio	1.1 ± 0.2	1.1 ± 0.1	1.2 ± 0.2	1.3 ± 0.1	NS
Specific growth rate	2.0 ± 0.4	1.5 ± 0.4	1.5 ± 0.1	1.1 ± 0.3	a ≠ d
Voluntary feed intake					
D01-D10	2.0 ± 0.1	2.0 ± 0.2	1.9 ± 0.1	2.3 ± 0.2	NS
D11-D20	2.0 ± 0.2	1.4 ± 0.4	1.7 ± 0.3	1.0 ± 0.3	*a* ≠ *b*, *a* ≠ *d*, *c* ≠ *d*
Decrease in voluntary feed intake between the first and last 10 days (%)	– 2 ± 10	29 ± 16	9 ± 13	57 ± 12	*a* ≠ *b*, *a* ≠ *d*, *b* ≠ *d*, *c* ≠ *d*
Fluoroquinolone (D11-D20) ingested (mg.kg^{-1}.d^{-1})		29 ± 8	35 ± 5		NS

For each parameter, significantly different results with different feeds are indicated (ANOVA, $p < 0.05$). NS : not significant at the 5 % level.

Table 3. – Growth and feed ingestion during the second trial. Feed demand, voluntary feed intake and feed refusals are given separately for each period of 10 days. The total amount of active ingredient ingested is also indicated. Data are shown as means ± SD (n = 3). The feeder, but for convenience all the preferred feeders (1) are in the left columns, and all the other feeders (2) are in the right columns.

Feeder	1	2	1	2
Feeding protocol				
D1-D10	*a*	*a*	*a*	*a*
D11-D20	*b*	*c*	*c*	*b*
D21-D30	*d*	*a*	*a*	*b*
Growth parameters				
Feed gain ratio	1.1 ± 0.1		1.3*	
Specific growth rate (% bw.d^{-1})	1.9 ± 0.1		1.8 ± 0.3	
Feed demand				
D01-D10	2.1 ± 0.1		2.0 ± 0.1	
D11-D20	1.9 ± 0.1		2.4 ± 0.7	
D21-D30	2.5 ± 0.2		3.3 ± 1.1	
Refusals (% feed demand)				
D01-D10	2.4 ± 2.0		1.7 ± 1.6	
D11-D20	2.2 ± 2.0		7.7 ± 6.9	
D21-D30	1.5 ± 0.5		4.0 ± 4.0	
Voluntary feed intake				
D01-D10	2.0 ± 0.1		2.0 ± 0.1	
D11-D20	1.9 ± 0.1		2.1 ± 0.5	
D21-D30	2.4 ± 0.2		3.1 ± 1.0	
Fluoroquinolone (D11-D20) ingested (mg.kg^{-1}.d^{-1})	37 ± 1		43 ± 10	

*Standard deviation is not given because some mortality was observed in two replicates.
No significant differences were observed in any parameter (ANOVA, $p < 0.05$).

Table 4. – Evolution of the proportion of feed demanded in the preferred feeder during the three phases of the second trial. Data are shown as means ± SD (n = 3). The feeder preference (% demand in the preferred feeder) was not related to the position of the feeder, but for convenience all the preferred feeders are labelled feeders 1, and all the other feeders are labelled feeders 2.

Feeder*	1	2	1	2
Feeding protocol				
D1-D10	*a*	*a*	*a*	*a*
D11-D20	*b*	*c*	*c*	*b*
D21-D30	*d*	*a*	*a*	*b*
Feeder preference (%)				
D01-D10	64 ± 5		68 ± 6	
D11-D20	65 ± 10		45 ± 12*	
D21-D30	52 ± 25		58 ± 9*	

*Indicate a significant difference between this period of 10 days and the previous one (ANOVA, $p < 0.05$).

diets and 35-40 % of the demands for the casein diet. The preference for fish meal *vs.* casein meal was greater with 78 % of the demands for fish meal.

More recently, Hidalgo *et al.* (1988), offered simultaneously five diets that differed only in methionine content to groups of sea bass, and showed that small individuals (mean weight = 2 g) used significantly more the feeder containing the 1.25 % methionine feed (which is very close to the optimum values, 1-1.30 %). During a similar experiment but with 200 g seabass, the choice of the self-feeder was apparently driven by the spatial location rather than by the methionine content of the feed (the location that allows the best survey of the surroundings). Cuenca *et al.* (1993), investigated the capacity of rainbow trout to discriminate between self-feeders distributing diets with normal or deficient levels of zinc. They demonstrated a clear zinc specific appetite in less than 6 days. A significantly higher velocity of discrimination between the normal and deficient zinc levels diet was also observed with initially zinc-deficient fish.

These studies demonstrate that fish can discriminate between self-feeders distributing different diets, but the spatial location of the feeders, or any other non-dietary factor, may also interact with the feeding behaviour and lead to pitfalls in data interpretation. In

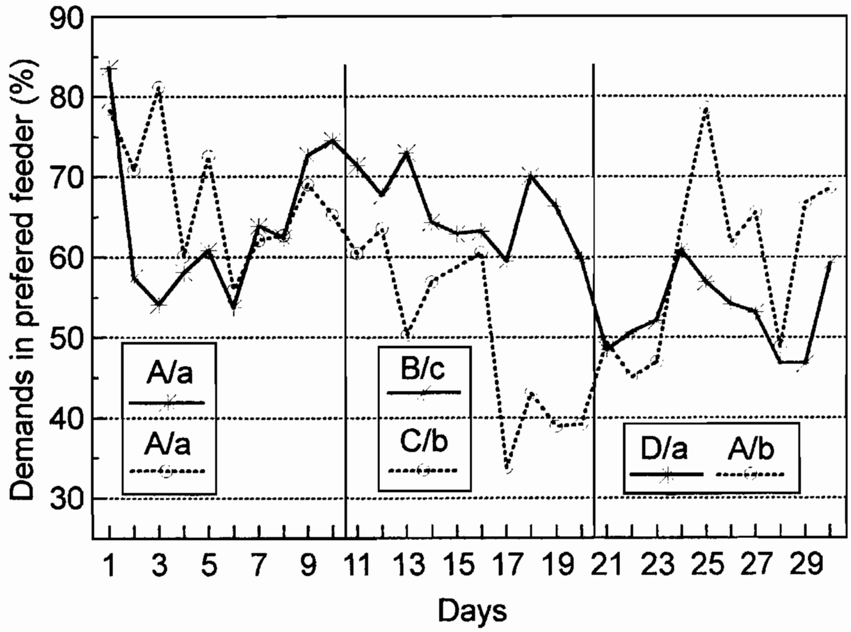

Figure 2. – Evolution of the percentage of demands in the preferred feeder during trial 2. Each curve represents the daily mean values of the triplicate groups. Superscript and lower case letter indicate the feed used to fill the preferred feeder and the other feeder, respectively. A technical failure did not allow recording of the feeding activity of fish submitted to the C/b protocol on D15.

our experiment with two feeders per group of fish, the first 10 days were used to determine such non-dietary influence on the choice of feeders loaded with the same diet, and during the next 10 days we studied the capacity of the fish to modify their feeder preference in relation to feed changes.

The fact that fish could discriminate between the feeds *b* and *c*, with a significant preference for feed *b* against feed *c*, is of considerable interest, since feed *b* and *c* contain the same concentration of the same active ingredient but under a different formulation. It seems that VFI is lowered with feed *b*, but feed *b* is very well accepted by the fish, because no increase in FR could be detected during trial 1, which was the case with feed *c*. Hustvedt *et al.* (1991), when using oxolinic acid, which is a first generation quinolone, at a concentration of 1 % in the medicated feed, also found a decrease in VFI. This reduction was similar (17 %) than what was observed in the present study (20 %).

With the aim of understanding the observed difference in feeding behaviour between fish fed with feed *b* and *c*, a short (four days) additional period of observation was performed with 12 groups of 28-30 fish issued from trials 1 and 2. These groups of fish were fed *ad libitum* by hand with diets *a, b, c* or *d*. Each day, VFI was reported and the feeding behaviour (essentially feed rejection) was observed. When fish where fed with feed *a* or *b*, pellets were never rejected before the satiety of the fish was reached. Conversely, pellets of feed *c* could be observed to be rejected and caught again more than 10 times, even at the beginning of feed distribution. Pellets were frequently abandoned, but the fish continued to catch new pellets that were distributed. When the fish were fed with feed *d*, pellets were almost never rejected. Nevertheless, it was obvious that fish were eating less and showed little excitement at the beginning of a new meal of feed *d*.

These observations could partially explain why the FGR was higher in some of the batches of fish fed with feed in comparison with the other batches. Indeed, in trial 1, tanks fed with diet *c* had a slightly higher FGR, associated with almost no decrease in voluntary feed intake but higher feed refusals than all the other batches of fish. In test 2, one tank showed also a high feed gain ratio associated with high feed refusals. One might hypothesise that in these tanks, an unknown part of the feed demand was reduced into powder by the fish. The amount of uneaten feed was therefore underestimated.

These observations lead us to the conclusion that the premix used in feed *c* is more detectable by the fish than the one used in feed *b* and leads to some feed refusals. Nevertheless, this decrease in feed intake was not very important, being lower than 20 % in almost all batches of fish fed with feed *b* or *c*. The decrease in appetite observed when fish are fed with feed *d*, associated with almost no refusals, indicate that sulfamerazine is not detected by the fish, but decreases appetite, exactly like an anorexic molecule.

Acknowledgements

The authors wish to thank Olivier Poupet for his technical assistance. These results were presented during the first COST 827 workshop on regulation of voluntary feed intake in fish, Aberdeen, 4-5 April 1997.

REFERENCES

Adron J. W., P. T. Grant, C. B. Cowey 1973. A system for the quantitative study of the learning capacity of rainbow trout and its application to the study of food preferences and behaviour. *J. Fish Biol.* **5,** 625-636.

Anthouard M., V. Wolf 1988. A computerised surveillance method based on self-feeding measures in fish populations. *Aquaculture* **71,** 151-158.

Boujard T., F. Médale 1994. Regulation of voluntary feed intake in juvenile rainbow trout fed by hand or by self-feeders with diets containing two different protein/energy ratios. *Aquat. Living Resour.* **7**, 211-215.

Boujard T., X. Dugy, D. Genner, C. Gosset, G. Grig 1992. Description of a modular, low cost, eater meter for the study of feeding behavior and food preferences in fish. *Physiol. Behav.* **52,** 1101-1106.

Bowser P. R., J. H. Schachte, G. A. Wooster, J. G. Babish 1990. Experimental treatment of *Aeromonas salmonicida* infections with enrofloxacin and oxolinic acid: field trials. *J. Aquat. Anim. Health* **2,** 198-203.

Choubert G., J. De la Noüe, P. Luquet 1982. Digestibility in fish: improved device for automatic collection of feces. *Aquaculture* **29**, 185-189.

Cuenca E. M., L.G. Diz, M. de la Higuera 1993. Self-selection of a diet covering zing needs in the trout. *In*: Fish nutrition in practice. S.J. Kaushik, P. Luquet eds. INRA eds. Paris, France, 413-418.

Hidalgo F., M. Kentouri, P. Divanach 1988. Sur l'utilisation du "self-feeder" comme outil d'épreuve nutritionnelle du loup, *Dicentrarchus labrax*- Résultats préliminaires avec la méthionine. *Aquaculture* **68,** 177-190.

Hustvedt S. O., T. Storebakken, R. Salte 1991. Does oral administration of oxolinic acid or oxytetracycline affect feed intake of rainbow trout ? *Aquaculture* **92,** 109-113.

Kentouri M., L. Léon, L. Tort, P. Divanach 1994. Experimental methodology in aquaculture: modification of the feeding rate of the gilthead sea bream *Sparus aurata* at a self-feeder after weighing. *Aquaculture* **119,** 191-200.

Kim J. D., S. J. Kaushik 1992. Contribution of digestible energy from carbohydrates and estimation of protein/energy requirements for growth of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* **105**, 161-169.

Paspatis M., T. Boujard 1996. A comparative study of automatic feeding and self-feeding in juvenile Atlantic salmon (*Salmo salar*) fed diets of different energy levels. *Aquaculture*, **145**, 245-257.

Poe W. E., R. P. Wilson 1989. Palatability of diets containing sulfadimethoxine, ormetoprim, and Romet-30® to channel catfish fingerlings. *Progress. Fish Cult.* **51,** 226-228.

Robinson E. H., J. R. Brent, J. T. Crabtree, C. S. Tucker 1990. Improved palatability of channel catfish feeds containing Romet-30®. *J. Aquat. Anim. Health* **2,** 43-48.

Schreck J. A., C. M. Moffitt 1987. Palatability of feed containing different concentrations of erythromycin thiocyanate to chinook salmon. *Progress. Fish Cult.* **49**, 241-247.