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Drugs and chemicals in aquafeeds: The problems and solutions

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Abstract. The purpose of this manuscript is to provide a general overview of current practices in the field of oral treatments. Special emphasis is placed on the technical peculiarities of premix and medicated feed technology that have important implications for in-feed medication in the aquaculture industry. On the other hand, there are a few promising developments in the field of novel oral delivery systems, i.e. bio-encapsulation and enteric-coated beads or biodegradable microspheres. Research in this area is still quite recent but large developments are expected in the future.

Keywords. Aquafeeds – Oral treatment – Medicated feed – Bioencapsulation – Microspheres.

Médicaments et produits chimiques dans les aliments d'aquaculture : Problèmes et solutions

Résumé. Cet article a pour objet de donner une description générale des pratiques actuelles en matière de traitements par voie orale. Une attention particulière est portée sur les spécificités technologiques des prémélanges et des aliments médicamenteux, spécificités qui ont des implications importantes pour les traitements via l'aliment en élevage aquacole. Enfin, il y a quelques développements prometteurs dans le domaine des nouveaux systèmes d'administration par voie orale, comme la bio-encapsulation, les microbilles protégées ou les microsphères biodégradables. La recherche dans ce domaine est relativement récente mais de grandes avancées sont attendues dans un futur proche.

Mots-clés. Aliments aquacoles – Traitement oral – Aliment médicamenteux – Bioencapsulation – Microsphères.

I – Introduction

Pharmacological research on aquaculture drugs has focused mainly on a few antibiotics widely used in aquaculture and, consequently, the development and commercialisation of new medicines for the treatment of aquatic diseases are rather scarce. There is an increasing demand for better knowledge of medicinal products and the availability of quality pharmaceutical and biological products suitable both for the prevention and treatment of diseases of cultured fish. In-feed medication has largely benefited from the progress made by the pharmaceutical industry on modern formulations, as well as some innovative oral delivery systems, such as bio-encapsulation and protected micro-diets.

Despite the fact that over 100 aquatic species are being farmed at a significant level around the world, most pharmacokinetic studies have been performed on only a few species, most notably in salmonid fish, as well as some other coldwater and or temperate species. However, there are considerable inter-specific variations in the pharmacokinetics of antibacterial compounds, so species-specific information is always needed before determining a suitable product to be used. Although Mediterranean fish culture has expanded during the last decade to become a prominent market, very little research has been undertaken so far on marine species, such as sea bass and sea bream. Flumequine and oxolinic acid have been the most studied compounds to date.

II – In feed medications: Characteristics, pro's and con's

The administration of medicines via the feed is generally the adopted method for treating a large

number of fish, whenever possible, because it is a much less wasteful way of administration than medication through the water. The most obvious limit to in-feed medication is that the fish to be treated must actually be feeding. Thus, it has no application to egg and sac-fry, nor for most reared species, because, except for Salmonids, the larvae and young fry need to be fed on live preys. Furthermore, fish suffering almost any kind of disease have a tendency to cease feeding. Thus in-feed medication is seen more as a prophylactic method than a therapeutic one: the goal is to medicate still healthy fish but that are in contact with diseased ones to prevent them contracting or expressing the disease. Already diseased fish will generally die irrespective of treatment.

Finally, the efficacy of an oral medication also depends on the drug concerned not being digested and transformed into inactive metabolites before absorption.

The preferred dosage form for aquaculture medicated feeds is a powdered premixture including the active compound and one or more excipients that can act as active carriers or as diluents for the active drug.

III – Development of premixes

Although premix manufacturing can apparently be as simple as mixing a pure chemical with a cheap diluent in order to sell it with higher profit margins, progress in premix technologies have allowed the appearance of efficacious products, genuinely adapted to oral administration. Some formulation and process technologies, commonly encountered in human pharmaceuticals, have found applications in aquaculture premix manufacturing.

The availability of new functional excipients and high-shear mixers and granulators has led to the development of very potent homogeneous premixes of active substances, even at low concentrations.

Manufacturing a medicated premix relies on the selection of functional excipients that should be compatible with the chemical used, and the design of a robust manufacturing process.

1. Regulatory aspects

In the European Union, premixes and medicated feeding stuffs are regulated by the Council Directive 90/167 (CEC, 1990). Addition of medicinal products to feeding stuffs in order to prepare a medicated feed can only be carried out by means of an authorized premix, although some exemptions do exist (CEC, 1990). The minimum concentration (w/w) of the premix in the compound feed must be 0.2%.

Premixes for use in veterinary medicated feeding stuffs are subject to a specific monograph in the European Pharmacopoeia: they are a separate pharmaceutical form, different from oral soluble powders or granules. The mixing properties of a premix with the feed ration can depend especially on the use of a particular type of compounder and associated blending parameters. Mixing studies must be undertaken in full production-scale batches to demonstrate a homogeneous distribution of the drug into the feed.

2. Formulation of premixes

Traditionally, premixes have long been formulated with natural vegetable carriers such as ground corncobs, toasted soy flour, or powdered minerals (vermiculite, clays and limestone). The use of these naturally occurring products has become questionable in GMP premix manufacturing plants because of their quality variations and potential heavy microbial or mould contamination. Consequently, they are usually replaced by compendial grade excipients acting as carriers for fine drug particles or as diluents for potent concentrated compounds. These include corn starch, maltodextrins, dicalcium phosphate, lactose or crystalline cellulose.

Maltodextrins are derivatives from partially hydrolyzed starch and have the advantage of being highly flowing products.

Another key parameter to be optimized in premixes is their dusting level, because the dust generated during the manufacture and packaging process and subsequent use by farm workers causes safety concerns. Various products can be used to lower the dusting potential of dry blend formulations. Mineral or vegetable oils are used at 1 to 3%, but can lead to agglomeration of particles especially at high levels of incorporation. Propylene glycols or polyethylene glycols of various molecular weights (PEGs) have a lesser agglomerating tendency but, on the other hand, they are subject to self-oxidation with time and, therefore, should not be used for drugs prone to oxidation. At levels of 3 to 4%, PEGs can reduce threefold the dust value of a starch/maltodextrin blend. At higher levels the flow ability of the mixture would be hampered.

3. Manufacturing of premixes

The basic equipments for processing premixes are mills and mixers/blenders. These can be low-shear traditional mixers like ribbon blenders, or high-shear mixer/granulators. High-shear devices fulfil a dual mixing and cutting action and are more efficient at homogeneous dispersal of compounds at very low concentrations (down to 0.1%) in the premix blend.

The major properties that should be achieved are the homogeneity of the drug in the finished feed, its compatibility with typical feedstuff and its stability after storage and shipping.

The homogeneity of the drug in finished feed needs to be measured on sufficient samples, both in terms of size and volume. Three samples are taken from the top, middle and bottom of the mix. The sample size should reflect the daily intake of the target species but without being greater than 50 g. The compatibility of the drug with all ingredients and additives of the feed (minerals, vitamins, antioxidants, etc.) needs to be assessed.

The stability of the drug in medicated feed must be assessed on three samples, preferably of commercial batch sizes. The fishfeed used should be intended for the target species and age group. Medicated feed should be packaged in the usual bags and stored under various test conditions of temperature, humidity and light or sun exposure. They should be transported to assess the effects of shipping and vibrations on the segregation of the drug from the pellets. The shelf life proposed for the medicated feed should be based on the results of these studies.

Eventually a properly designed premix for aquaculture should bear all the properties described in Table 1.

Table 1. Properties of a high quality aquaculture premix (modified after Shao, 2001)

Parameter	Quality	Comments
Physical	Elegant product, free-flowing and dust free Good physical stability. No segregation during shipping / vibration Non-hygroscopic, non-electrostatic	Easy to measure; low exposure to workers
Chemical	Good stability of active ingredient in both premix and medicated feed Homogeneity of active ingredient in premix and medicated feed	Compatibility of drug with excipients in the premix and ingredients of the feed
Efficacy	Good bioavailability and general efficacy	
Environment	Environmentally friendly. Rapid degradation in aquatic systems, short half-life in water and sediments	Low impact on non-target organisms in aquatic environment
Economic	Economic to manufacture	

IV – Incorporation into feed

In terms of volume, the daily dose of medicinal product should be contained in a quantity of feed corresponding to at least half the daily feed ration distributed.

There are several methods for incorporating this dose into the feed stuff. The premix can be incorporated at the beginning of the manufacturing line (pelleted medicated feeds) or it can be added at the end of the pelleting process, after granulation or extrusion. In this case the premix is poured or sprayed on the surface of the pellets (surface-coated medicated feeds).

In order to achieve optimal homogeneity of the drug concentration, the ideal way to mix the drug is to add it at the beginning of the premix process, added to the blend together with other micro-ingredients, just prior to pelleting. Obviously, this can only be done at the feed mill, both on press granulation lines or on extrusion lines. However, the pelleting process involves high temperature and humidity conditions and hence this method can be used only with heat stable compounds.

On the other hand, the major drawback of this "initial incorporation" is the risk of carry over along the whole manufacturing line and of subsequent cross contamination of blank feeds with medicinal drugs (see next section). An intermediary option would be to have a specific pelleting line fully dedicated to medicated feeds, but this is quite an exceptional situation, almost restricted to Norway. In any case, this method can only be considered for very large batches of medicated feeds because the whole production line must be thoroughly cleaned after producing a batch of medicated feed.

Surface coating is the only suitable way to medicate feeds with products that don't resist extrusion (especially heat labile compounds). It is also the only affordable way to produce small volumes of medicated feeds. This can be done either at the feed mill or on-farm.

At the feed mill, the drug can be added after extrusion and pre-cooling, in the fat coater. Modern vacuum coaters allow the addition and adequate mixing of very small quantities of additives in liquid or powder forms.

On fish farms, the mixing is generally done in a concrete mixer: the pellets are loaded first and the powdered drug is poured and thoroughly mixed to the feed, followed by a binding agent. In most cases this binding agent is an edible vegetable or fish oil such as sunflower oil or cod liver oil. The medicated feed is then allowed to stay until the pellets have absorbed the added oil.

The biggest obstacle to surface coating is that it is difficult to achieve a sufficient homogeneity of the mixture. This is the reason why administrative authorities in most countries require it to be made only in registered facilities, even if it is a simple concrete mixer (samples must be taken and analysed in order to check the homogeneity of the mix). Other important disadvantages include leaching and palatability problems (see below).

V – Major problems for pelleted feeds: Carry over and cross contamination

Carry over is an amount of product that during production remains in the production line and comes through into the subsequent batch(es), thus carrying the risk of contamination. Concerns arising from problems of carry over and cross contamination are not specific to veterinary drugs, but also apply to various essential additives like vitamins, provitamins or pigments. Mixing of various solids is the first step in feed compounding, but disperse solid mixtures are relatively unstable, due to structure differences of the ingredients and insufficient binding forces between them. The micro-components especially tend to segregate, resulting in mixture heterogeneities, losses of nominal content, carry over of residues in the processing line and cross contamination of the following batches. Carry over can occur during feed processing, handling, or delivery.

1. Regulatory aspects

There is no zero-tolerance, nor is there any legally fixed quantitative limit for carry over and cross contamination because absolute avoidance of carry over is technically impossible in compound feed manufacturing. However, the European Union has set up regulatory guidelines requiring that technical tools and organisational measures be used in order to minimize cross contamination and to avoid errors as much as possible.

In some cases, cross contamination can cause serious problems. For example, carry over of monensin from cattle feed to horse feed may kill the horse (Harner III *et al.*, 1996). Acceptable limits can be deducted from the maximum residue level (MRL) values and the feed suppliers should follow the recommendations for Good Manufacturing Practices edited by the European Feed Manufacturers Federation (FEFAC).

2. Practical situation

As an example, internal quality standards can be set so that carry over to the subsequent batch should be minimized to less than 4% to 7% for a compound feed (depending on the length of the tested production line) and less than 1% for a premix plant. According to investigations carried out by a German feed research institute on more than 350 feed mills, these levels are difficult to respect, as the average contamination rates of a following batch in feed mills can vary between 4 and 10% for compound feeds and between 1 and 5% for premixes (Fig. 1) (Heidenreich, 1997). For blend homogeneity, the coefficient of variation should be less than 5%, although between 5 and 10% is still acceptable.

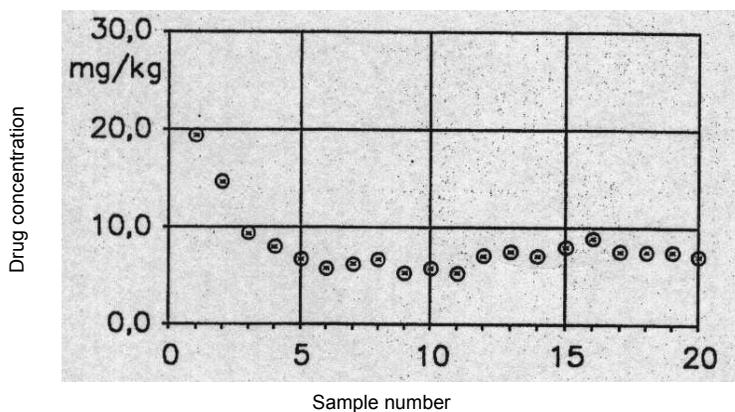


Fig 1. Carry over - indicator concentration in the first rinsing batch (target feed indicator concentration = 100 mg/kg) (after Heidenreich, 1997).

Once carry over has been controlled and minimized, the risk of contamination can be obviated only through production sequencing, flushing, and complete cleaning out of feed processing and delivery equipment. After manufacturing a medicated feed, one rinsing batch must be used as a minimum. However, these measures can not compensate for every type of carry over effect. Eventually, the only way to completely avoid cross contamination of non-medicated feeds consists in the manual admixing of the medical substance at the end of the production line, using a small dedicated high performance mixer.

VI – Major problems for surface coated feeds: Leaching and palatability

1. Leaching

Leaching of drug into the water will occur with all kinds of medicated feeds, but is especially high with surface coated pellets. The extent of leaching depends on various factors including the water solubility of the chemical, the time during which the feed remains in the water before the fish eat the pellets, and the size of the pellets (ratio of surface area to weight). The smaller the pellets the higher will be the leaching losses.

A study by Rigos *et al.* (1999) showed that pellets (4.5 x 6 mm) oil-coated with oxolinic acid (OA) and oxytetracycline (OTC) were considerably affected by leaching at 16°C (losses of 55.5% and 42.5% for OA and OTC, respectively) and at 24°C (32% for OA and 47% for OTC). Significantly fewer losses occurred with "on line" mixed pellets (5% for OA and 6.5% for OTC at 16°C; 10% and 20% at 24°C, respectively). The reason why high temperatures had a strong influence on the leaching of both drugs in mixed pellets but not in the surface coated ones was not explained.

2. Palatability

Depressed palatability is an important drawback of medicated feeds that can originate from the chemical itself, from the excipients or from the binders used to medicate the feed. Bearing in mind that already diseased fish have the tendency to stop feeding, this problem can be of crucial importance for the success of treatments. Several fish species, notably flatfish, are particularly delicate and fussy. Even Mediterranean species like sea bass sometimes hardly consume feed if it is not attractive enough. For example, in the same study, Rigos *et al.* (1999) showed that surface coated oxytetracycline was highly repellent for sea bass and depressed feed consumption by 90%!

3. Top coating binders

A way to obviate leaching and palatability is to top coat the medicated feed with a special binder designed to overcome these problems. A binder based on an alginate matrix, TOPGEL® (PROVIMI-VETCARE), was initially developed for shrimp feeds. As such, it has high binding properties in order to keep the drug intact after several hours in the water. It also contains strong attractants, and has found interesting applications in fish feeds. Another binder, MEDI-TAK® Oil, made from a mixture of stabilised poly-unsaturated fatty acids, was also put on the market in 1996 (Treves-Brown, 2000).

VII – Bioavailability of drugs

1. Definition of bioavailability

The bioavailability (F%) of a drug represents the fraction of an oral dose which is effectively absorbed into the circulatory system. It is determined by comparing blood levels following single oral and intravenous administration of the drug. For premixes, bioavailability should be determined by administration of a complete medicated feed prepared following the procedure recommended by the manufacturer.

The blood concentrations are estimated by the AUC (Area under the concentration/time curve) and F is calculated according to the following equation:

$$F\% = \left[\frac{\text{AUC oral} \times \text{dose intravascular injection}}{\text{AUC intravascular} \times \text{dose oral administration}} \right] \times 100$$

2. Bioavailability in sea water

Of particular concern for sea farming is the reduced bioavailability of many antibiotics in seawater compared to freshwater. Notably, quinolones and tetracyclines are known to bind with seawater borne divalent cations Mg^{2+} and Ca^{2+} ions, especially Mg^{2+} (Lunestad and Goksøyr, 1990; Pye-MacSwain *et al.*, 1992; Barnes *et al.*, 1995; Smith *et al.*, 1996). Normal seawater concentrations of Mg^{2+} have a marked effect on both antibacterial activity and uptake by fish of various antibiotics. Barnes *et al.* (1995) found the minimum inhibitory concentration (MIC) of oxolinic acid against *Aeromonas salmonicida* to be increased 40- to 60-fold with seawater Mg^{2+} . Of course, such an effect is particularly important when the feed is medicated by surface oil coating.

Whatever the bioavailability of the chemical used, an important point is that in-feed medication is prophylactic rather than therapeutic and this has profound implications on the desired kind of pharmacokinetic profiles (Treves-Brown, 2000). High concentrations of drug are not necessarily needed in the organs and tissues affected by the disease but rather at normal portals of entry of the pathogen. The major portals of entry for pathogens are the gills and the gut, and to a lesser extent the skin. Seawater fish drink continually, so the gut wall is an important portal of entry for many marine pathogens. If entry is through the gut wall, then even the portion of the drug which will stay in the gut lumen without being absorbed can play an important local role to prevent ingress of pathogens.

VIII – Seawater pharmacokinetic profiles of antibiotics: Two examples

There are considerable inter-specific variations in the pharmacokinetics of antibacterial compounds, so species-specific information is always needed before determining the suitable product to be used. Most pharmacokinetic studies on antibiotics commonly used in aquaculture have been performed on salmonid fish, as well as some in other coldwater and/or temperate species. Although Mediterranean fish culture has expanded during the last decade to become a prominent market, very little research has been undertaken so far on marine species, such as sea bass and sea bream. Most studied compounds are flumequine and oxolinic acid.

1. Oxolinic acid

Oxolinic acid (OA) is a licensed antibiotic in several European Union member countries that displays a broad spectrum of antibacterial activity, especially against Gram negative bacterial fish pathogens (Ledo *et al.*, 1987). As such, it has been used extensively to treat a number of systemic bacterial diseases in a variety of fish species.

The absorption of OA seems to be longer in marine fish like sea bass (0.69 h) (Poher *et al.*, 1997), and Atlantic halibut (7 h) (Samuelsen and Ervik, 1999) than in freshwater fish like rainbow trout. In gilthead sea bream (*Sparus aurata* L.), the elimination half-life time is the shortest reported for any farmed species. Distribution and elimination of the drug are rapid: the distribution half-life and elimination half-life times are 0.51 and 12.60 h, respectively (Rigos *et al.*, 2002a). This means that the initial drug penetration from the plasma to the tissues is rapid and adequate.

Following oral administration, the bioavailability (F%) of OA (at 30 mg/kg) is low (14%) but comparable to other species like Atlantic halibut (15%) (Samuelsen and Ervik, 1999) and turbot (27.9%) (Poher and Blanc, 1998). The absorption of OA can also be reduced by the unfavourable intestinal pH of marine fish: at $pH > 8$ the major part of the drug is in its ionized form and is not absorbed. Maximum levels are observed in muscle after 16 h and muscle concentrations remain higher than plasma. The fast elimination of OA suggests short withdrawal times for human consumption.

Some recent advances have been obtained with OA administered in the form of a carbitol ester which increases the lipophilic character of the drug: the bioavailability and plasma levels were considerably increased (Samuelsen and Ervik, 1999). Ultra-fine forms of OA have also been proven to improve its bioavailability because of enhanced dissolution resulting from micronization.

2. Flumequine

Flumequine is another member of the quinolone family displaying a very broad bactericidal activity which is widely employed in fish therapy because it has a relatively low minimum inhibitory concentration (MIC) against important bacterial fish pathogens and also has attractive pharmacokinetic properties. It has been demonstrated in Atlantic salmon held in seawater that flumequine is eliminated at substantially higher rates than in freshwater (Sohlberg *et al.*, 2002).

In sea bass kept at 18°C, the absorption and elimination of flumequine are fast. Rigos *et al.* (2002b) calculated an absorption half life ($t_{1/2\alpha}$) of 1.05 h, indicating a relatively rapid absorption compared to salmonids. The elimination half life ($t_{1/2\beta}$) was 10.71, indicating a fast elimination from plasma. The clearance of the drug is higher than other studied species and, like OA, the withdrawal period can be short.

Further research is needed to study the pharmacological profiles of these antibiotics, notably their absorption, tissue distribution and residue depletion following multi-day oral treatment, under practical farming conditions.

IX – Oral antiparasitic agents

1. Sea lice therapies

Ivermectin was the first oral drug that proved to be very effective against sea lice. Salmon farmers of several countries including Ireland, UK, Canada and Chile have commonly used it for many years, although it was never licensed for use in fish (Høy *et al.*, 1992).

Ivermectin has also been investigated in the Mediterranean on sea bass and gilthead sea bream for treating isopod (*Anilocra physodes*) and copepod infestations (*Caligus* sp. and *Lernanthropus kroyeri*). Oral ivermectin treatments at 0.5 mg/kg proved highly efficacious in controlling such crustacean infestations on sea bass. However, the drug showed some toxicity at higher concentrations or at decreased temperatures (Athanassopoulou *et al.*, 2002).

More recently, a new easier to use avermectin, emamectin benzoate (Slice® - Schering Plough) was developed and has obtained a Marketing Authorisation in Norway and the UK. It is very potent against copepod infestation and is relatively non-toxic to salmonids. Its safety and efficacy for treating Mediterranean parasites has not been investigated.

Among another valued family of compounds acting as chitin synthesis inhibitors some products have gained temporary approval in certain Northern European countries for oral use against sea lice, including diflubenzuron (Lepsidon® - Ewos) and teflubenzuron (Ektobann®/Calicide® - Nutreco). These compounds are given by the in-feed method, but have the problem of binding to marine sediments, thus creating potential environmental concerns.

2. Other antiparasitic agents

Anthelmintic agents are drugs used to control roundworms (Nematodes), tapeworms (Cestodes) and flukes (Trematodes).

Praziquantel (a pyrazinoisoquinoline) is registered in Norway for use against intestinal tapeworms. It is administered in feed with a withdrawal period of 14 days. Albendazole and

fenbendazole are currently being investigated for use against flukes and larval tapeworms in salmon.

X – Conclusion

The technological characteristics of oral medicine products are of utmost importance to ensure the effectiveness of in-feed medication in the aquaculture industry. Furthermore an accurate knowledge, of the species-specific pharmacological peculiarities and of the impact of the environment (seawater) on the physical properties of drugs is of equal importance. Pharmacokinetics and pharmacodynamics studies are therefore essential before predicting the success of an oral treatment.

For particular applications like the treatment of young larvae and fry, some success have been obtained with the bio-encapsulation of drugs in live feeds, especially with artemias. Other innovative ways of oral delivery like microspheres or coated beads offer the possibility to protect fragile molecules like the antigens of oral vaccines, from deterioration by gastric juices and to carry them up to their target sites in the intestine. Though still quite recent, large developments are expected from these innovative technologies in the near future.

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