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Praziquantel form, dietary application method and dietary inclusion level affect palatability and efficacy against monogenean parasites in yellowtail kingfish

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ABSTRACT: The bitterness of racemic praziquantel (PZQ) currently constrains its use as an in-feed treatment against monogenean flukes in finfish aquaculture. In an effort to increase the palatability of diets containing racemic PZQ for yellowtail kingfish, the palatability and efficacy of 2 forms of racemic PZQ (powder or powder within microcapsules) against natural infestations of skin and gill flukes were compared using 2 different dietary application methods (incorporated within the pellet mash prior to extrusion or surface-coated after extrusion) at active dietary inclusion levels of 8, 16 and 25 g kg^{-1} in large (3.5–4 kg) yellowtail kingfish. There was no clear benefit of incorporating PZQ into diets prior to extrusion. PZQ microcapsules improved the palatability of PZQ-containing diets but did not completely mask the bitter flavour. At the lowest active dietary inclusion level of 8 g kg^{-1}, ingestion of the diet containing PZQ microcapsules was equal to the control and significantly better than that containing PZQ powder. At an inclusion level of 16 g kg^{-1}, ingestion of the PZQ microcapsule diet was significantly better than that containing the same inclusion of PZQ powder but significantly lower than the control. Consumption of the diet containing 25 g kg^{-1} of PZQ microcapsules was poor. All fish consuming medicated feeds had a significant reduction in flukes relative to control fish; however, efficacy data and blood serum analysis suggested that diets containing PZQ microcapsules had lower bioavailability than those containing PZQ powder.

KEY WORDS: Praziquantel · In-feed · Medicated diets · Yellowtail kingfish · Monogenean · Fluke management · Anthelmintic

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INTRODUCTION

A number of *Seriola* species are being commercially cultured or investigated for culture throughout the world, including in Japan (Nakada 2000), Australia (Booth et al. 2010), New Zealand (Poortenaar et al. 2001), the Americas (Benetti et al. 2004) and within Europe (Abbink et al. 2012). Throughout their distribution, and including farms in all aforementioned regions, all species are susceptible to infestations by monogenean parasites, including the polyopisthocotylean gill fluke *Zeuxapta seriolae* and the monopisthocotylean skin flukes *Benedenia seriolae* and *Neobenedenia girellae* (see Tubbs et al. 2005, Hirayama et al. 2009).

These monogenean parasites have been identified as significant risks to the sustainability and profitability of *Seriola* culture industries (Hutson et al. 2007). In Australia, treatment of monogenean parasites in *Seriola* involves routine bathing in hydrogen peroxide (Ernst et al. 2005, Mansell et al. 2005), a process that is labour intensive, logistically challeng-

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ing and that can result in significant mortality if not correctly implemented. Such treatments are also expensive, with estimates that treatment and other associated management costs contribute up to 20% of the cost of production (Ernst et al. 2005). Without treatment, such flukes can lead to anaemia, loss of appetite, poor growth, secondary bacterial infections and mortality (Grau et al. 2003, Mansell et al. 2005).

Praziquantel (PZQ) is a broad-spectrum anthelmintic drug that has been used therapeutically in animals and humans for over 30 yr (Andrews et al. 1983) and whose efficacy against a wide range of monogenean flukes has been demonstrated across a range of fish species (Schmahl & Mehlhorn 1985, Buchmann 1987, Schmahl & Taraschewski 1987, Thoney 1990, Santamarina et al. 1991). Bath treatments of 2.5 mg PZQ l^{-1} for 48 h, for example, are highly effective against both *Z. seriolae* and *B. seriolae* in *S. lalandi* (see Sharp et al. 2004, Tubbs & Tingle 2006); however, this method of administration is expensive for sea cage operations and results in the discharge of large quantities of PZQ into the environment. In-feed treatments represent a more cost-effective delivery route and doses of between 50 and 800 mg kg^{-1} d^{-1} for up to 20 d have been reported with varying degrees of success (Tojo & Santamarina 1998). The oral administration of PZQ is approved for use against *B. seriolae* in Japan (Hadaclean®, Bayer Animal Health and Suisanyo Benesal®, Kyowa Hakko); however, there is little data on its efficacy. Issues relating to the palatability of PZQ have been reported and constrain its use. PZQ is known to be very bitter (Meyer et al. 2009), and appetite suppression and diet rejection has been reported in *S. quinqueradiata*, *S. dumerili* (see Hirazawa et al. 2004) and *S. lalandi* (see Williams et al. 2007). If palatability problems can be overcome, PZQ has great potential as a cost-effective and easily administered treatment against such flukes. Methods suggested by Williams et al. (2007) to improve the palatability of PZQ for *Seriola* included microencapsulation, flavour masking and incorporation of PZQ directly within manufactured pellets.

This study comprised 2 experiments to compare the effects of PZQ form, dietary inclusion method and dietary inclusion level on diet palatability and efficacy in large yellowtail kingfish naturally infested with skin or gill flukes. The 2 forms of PZQ tested were racemic PZQ powder and racemic powder incorporated into microcapsules. The 2 dietary application methods involved either direct incorporation of each PZQ form into the feed mash prior to extrusion into feed pellets or by gelatine-coating each form onto the exterior of the feed pellets after extrusion. Dietary inclusion levels of 8, 16 and 25 g of active PZQ per kilogram of food were tested. Large fish were used in this study, as their lower feed intake rates necessitate higher dietary inclusion levels to achieve effective dose rates, and they therefore represent the greatest challenge in terms of diet palatability. The highest dietary inclusion of 25 g kg^{-1} was chosen as it represents the likely upper range of dietary inclusions necessary to achieve effective dose rates to treat skin flukes in large fish in cool water.

MATERIALS AND METHODS

Trial 1

Treatment diets outlined in Table 1 were prepared from a commercial yellowtail kingfish mash containing 45% protein and 20% lipid (Ridley Agriproducts; www.ridley.com.au). A single batch of 0.5 tonnes of this mash was split into 3 portions: one of 0.3 tonnes and 2 of 0.1 tonnes each. To the 2 smaller portions, either 8 g kg^{-1} of racemic PZQ powder (99.42%, TNN Development Company) or 20 g kg^{-1} of microcapsules containing 40% racemic PZQ (Zamira Life Sciences) were added, yielding 8 g kg^{-1} of active PZQ in both portions. The third portion of mash contained no PZQ. All 3 portions were extruded into 9 mm diameter pellets using a Wenger X-85 extruder (www.wenger.com) at the Australasian Experimental Stockfeed Extrusion Centre, Roseworthy, South Australia. After extrusion, the non-medicated portion was split into 3 equal portions. Each portion was gelatine-coated with no PZQ (control), PZQ powder or PZQ

Table 1. Treatments investigated in Trials 1 and 2. PZQ: praziquantel

Treatment	PZQ form	Inclusion method	Active PZQ inclusion (g PZQ kg^{-1} diet)
Trial 1			
1	Powder	Surface-coated	8
2	Microcapsules	Surface-coated	8
3	Powder	Mash	8
4	Microcapsules	Mash	8
5	Control	–	0
Trial 2			
1	Powder	Surface-coated	16
2	Microcapsules	Surface-coated	16
3	Microcapsules	Surface-coated	25
4	Control	–	0

microcapsules at the aforementioned active inclusion level of 8 g kg^{-1} to the latter 2 diets. To coat the pellets, 25 g of gelatine (Davis Gelatine, New Zealand) was dissolved in 625 ml of hot tap water (50°C) on a magnetic heater/stirrer. Once dissolved, this gelatine solution was poured into a rotating cement mixer containing 25 kg of pellets and the necessary quantity of PZQ. Mixing continued for a further 5 min to ensure a homogeneous coverage of gelatine and PZQ. Pellets were then removed from the mixer, spread in a thin layer and placed in a cool-room at 8°C to allow the gelatine to set. Once set, pellets were returned to feed bags until use.

This trial was conducted at the Australian Centre for Applied Aquaculture Research, Fremantle, Western Australia. Forty yellowtail kingfish naturally infested with gill flukes were randomly selected from a holding tank containing 300 fish and distributed evenly into each of five 5 m^3 tanks. Seawater with a temperature of 22.5°C flowed through each tank at a rate of 20 l min^{-1}. In each tank, a vertical water inlet manifold and central aeration (similar to that described by Partridge et al. 2006) rapidly moved any uneaten food and faeces to the centre drain, where they were removed by opening this drain after each feed. During a 6 d acclimation period to these tanks, fish were fed to satiety on the control diet once per day. The average food intake during this acclimation period was used to calculate the fixed ration of food offered to each tank during the trial. On Day 7, feed was switched to the 5 experimental diets and feeding continued for a further 7 d. Fish were fed slowly to ensure very few pellets remained uneaten. The actual amount of food consumed was recorded for each tank. Each trial was repeated 3 times to provide replication through time, with different fish used for each replicate (i.e. the same fish were never used twice). The average weight of fish was 3.49 ± 0.03 kg. There was no significant difference in fish size either between tanks or between replicates over time.

Trial 2

Based on the results of Trial 1, Trial 2 utilised only diets surface-coated with the same 2 forms of PZQ and higher active dietary inclusion levels of 16 and 25 g kg^{-1} (Table 1). Only diets coated with PZQ microcapsules were tested at the highest inclusion level. Commercially available 9 mm yellowtail kingfish pellets (Ridley Agriproducts) were gelatine-coated as previously described with PZQ powder or PZQ microcapsules at the required rates to achieve the aforementioned active dietary inclusion levels. The control diet containing no PZQ was again coated with gelatine.

This trial was conducted at the Batavia Coast Maritime Institute, Geraldton, Western Australia. Twelve 4 m^3 tanks were used to test the 4 treatments outlined in Table 1 in triplicate. Each tank was stocked with 5 yellowtail kingfish averaging 3.95 ± 0.09 kg in body weight and naturally infested with skin flukes. Seawater with a temperature of 23.2°C flowed through each tank at the rate of 30 l min^{-1} using the same aforementioned tank hydrodynamics. Fish were acclimated to the experimental system for a period of 4 d during which time they were fed to satiety once daily on a control ration containing no PZQ. The average food intake during this acclimation period was used to calculate the fixed ration of experimental diets offered during the next 7 d. Feeding was again conducted slowly to ensure that the vast majority of pellets added to the tank were consumed. Food consumption and the time taken for fish to consume their ration were recorded for each tank.

Sampling and data analysis

Quantification of the enatiomeric ratio of both forms of PZQ was undertaken by the Chemistry Centre of Western Australia using a method accredited by the National Association of Testing Authorities, Australia (NATA). PZQ microcapsules were ultrasonicated and solublised in methanol at room temperature. PZQ powder was lightly ground using an agate mortar and pestle before methanol extraction. Samples were diluted and analysed using a Waters Alliance 2695 HPLC with separation achieved using a chiral lux column and quantification with a photodiode array detector.

Daily food intake for fish in each replicate was expressed as a percentage of that eaten by fish in the control treatment. Based on the actual intake rates, an average daily dose of PZQ (mg PZQ kg^{-1} wet fish body weight [BW] d^{-1}) was calculated. For Trial 1, a 2-way ANOVA on arcsine-transformed data was used to determine the effect of PZQ type and application method on ingestion and dose rate received. Where significant differences were detected, Tukey's honestly significant difference (HSD) test was used to compare least square means. As only one dietary inclusion method was investigated in Trial 2, arcsine-transformed ingestion, dose rate and time taken to consume diet in this trial were analysed by 1-way ANOVA followed by Tukey's HSD test.

On Day 8 of each experiment, fish were anaesthetised, weighed and sampled for flukes using a method modified from Williams et al. (2007). All fish were firstly anaesthetised (30 mg l^{-1} AQUI-S, www.aqui-s.com) and then transferred to a 150 l bath containing dechlorinated tap water for 5 min to remove *Benedenia seriolae* and then to a 150 l bath containing 5 mg l^{-1} PZQ for 10 min to remove *Zeuxapta seriolae*. Water from each bath was then concentrated through a 50 µm mesh to collect flukes, which were then counted under a dissecting microscope. The prevalence and intensity of fluke infection were not determined prior to feeding treatment diets.

The percentage reduction in flukes in each trial was calculated according to Stone et al. (1999) as follows:

$$\%\text{Reduction} = 100 - \left(100 \times \frac{\text{Mean of treated replicates}}{\text{Mean of control replicates}}\right)$$

In Trial 1, a 1-way ANOVA was firstly used to compare the number of flukes on control fish against fish receiving medicated diets. A 2-way ANOVA was then used to determine the effect of PZQ type and application method on differences in arcsine-transformed percentage reduction data. Where significant differences were detected, Tukey's HSD test was used to compare least square means. In Trial 2, 1-way ANOVAs were used to compare the differences in fluke numbers between treatments and in arcsine-transformed percent reduction data between treatments, followed by Tukey's HSD tests. On Day 8 of Trial 2, blood was collected from 2 anaesthetised fish per tank, 2 h after offering a final feed of the treatment diet. Heparinised blood was then centrifuged at 10 000 rpm (7200 × *g*) for 10 min to separate plasma from red blood cells. Equal volumes of plasma from the 2 fish within each replicate was pooled and then frozen for later analysis of PZQ by the Chemistry Centre of WA using a NATA accredited method. Prior to analysis, samples were homogenised and a subsample was accurately weighed and then extracted into methanol. The sample was then diluted and analysed on an Agilent LCMS/MS using a C18 eclipse column, with the mass spectrometer operating in the ESI Positive mode. Three transitions were monitored and quantitation was carried out against a 5-point calibration of an authentic standard material.

Given the very short residence time of PZQ in the blood stream (Tubbs & Tingle 2006), we assumed that the PZQ measured in the blood was only derived from the final feed that occurred 2 h prior to the blood being collected. From the dietary PZQ inclusion level and the amount of food actually ingested during this feed event, the actual amount of PZQ ingested per kilogram of fish biomass was calculated and plotted against the measured PZQ concentration in the blood for each treatment.

Scanning electron microscopy was conducted on innate PZQ microcapsules and on a sample of these microcapsules gently extracted from within the extruded diets containing these capsules. Samples were mounted on gold disks and coated with 90 nm of gold on a Balzers Union Sputtering Device before imaging on a Philips XL20 scanning electron microscope (Philips Electronics N.V.).

RESULTS

Both forms of PZQ were confirmed to be racemic, with the microcapsules having an *S*-(+) to *R*-(–) ratio of 49.9:50.1 and the powder 50.1:49.9.

Trial 1

Based on the food intake during the acclimation phase, fish were offered a fixed ration of 0.80% BW d^{-1} during the trial period. Ingestion of each medicated diet as a percentage of that ingested in the unmedicated control treatment is shown in Fig. 1. Two-way ANOVA revealed no significant effects of PZQ type ($p = 0.12$) or application method ($p = 0.47$) on relative ingestion, but a significant interaction ($p = 0.0016$) between the two (Fig. 1). While PZQ microcapsules resulted in better ingestion in surface-

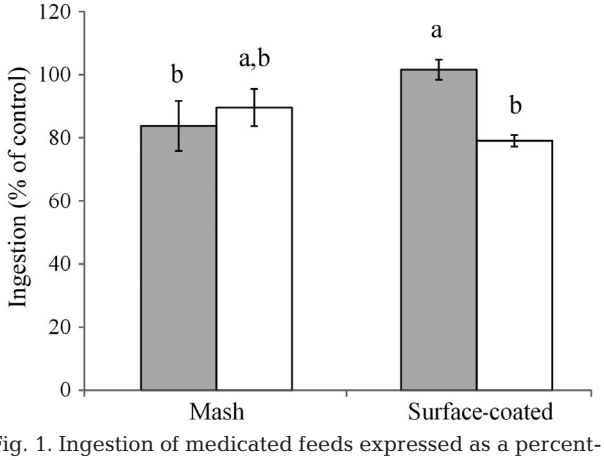

Fig. 1. Ingestion of medicated feeds expressed as a percentage of the food eaten by yellowtail kingfish receiving an unmedicated control diet. Active praziquantel (PZQ) dietary inclusion level 8 g kg^{-1}. Data are means ± SE. Columns sharing the same letter are not significantly different. ▒: PZQ microcapsules; □: PZQ powder

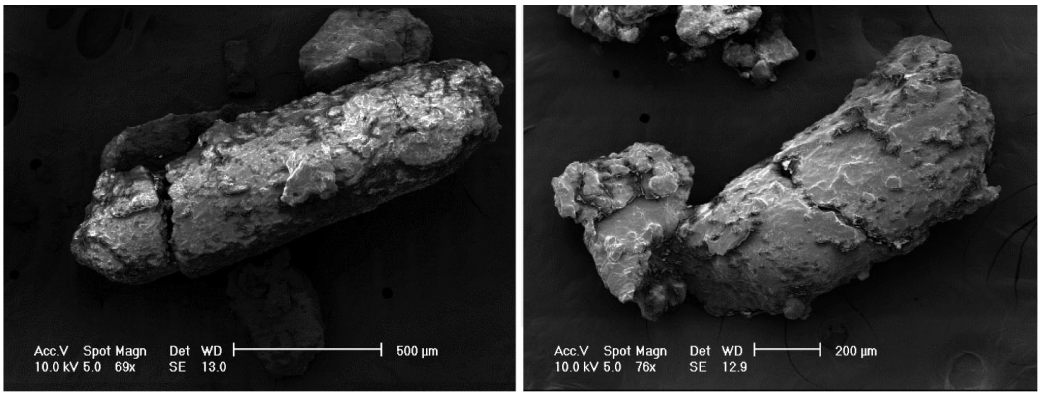

Fig. 2. Cracked microcapsules extracted from within dietary pellets into which they had been incorporated prior to extrusion

coated diets compared with PZQ powder, the opposite was seen for mash diets. Those fish fed the diet surface-coated with PZQ microcapsules ate 102 ± 3% of the unmedicated control, an amount significantly higher than that consumed by fish fed the diet surface-coated with PZQ powder (79 ± 2%) and those fish offered the diet containing PZQ microcapsules within the mash (84 ± 8%). Fish offered the PZQ powder within the mash consumed 90 ± 6%, which was not significantly different to any of the other diets. Scanning electron micrographs of the PZQ microcapsules that were incorporated within the mash prior to extrusion show them to be cracked and damaged (Fig. 2). Given the relationship between intake and dose, the outcome of the 2-way ANOVA for dose rate was the same as that described above for ingestion. Those fish fed the diet surface-coated with PZQ microcapsules received a dose of 63.4 ± 0.1 mg kg^{-1} d^{-1}, a rate significantly higher than that received by fish fed the diet surface-coated with PZQ powder (51.2 ± 1.6 mg kg^{-1} d^{-1}) and those offered the diet containing PZQ microcapsules within the mash (53.7 ± 5.0 mg kg^{-1} d^{-1}).

Fish in Trial 1 were naturally infested only with gill flukes. Those fish receiving the control treatment had 100% prevalence of infection and an average of 175 ± 16 flukes per fish at the completion of the trial, significantly more than fish receiving medicated diets (1-way ANOVA, p = 0.007). The percentage reduction in fluke numbers ranged from 80 ± 14% to 100%, with 2-way ANOVA showing no significant effects of PZQ form or application method on percentage fluke reduction (Fig. 3). Despite this, it is noteworthy that it was fish receiving diets surface-coated with PZQ powder that had 100% fluke elimination and not those receiving diets surface-coated with PZQ microcapsules, despite the former receiving a significantly lower dose of PZQ than the latter.

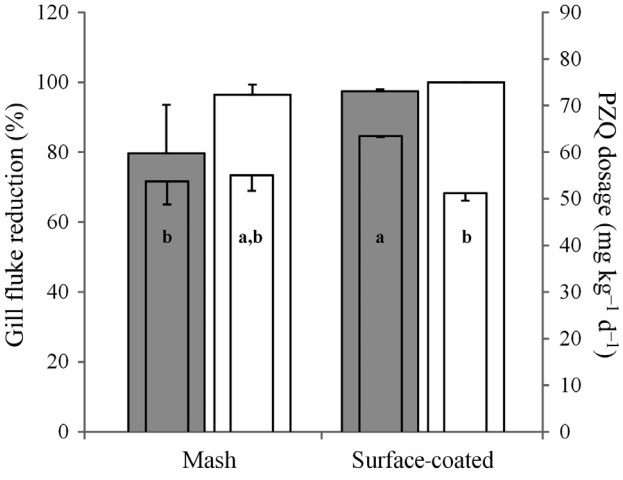

Fig. 3. Percentage reduction in gill fluke numbers relative to the control treatment in yellowtail kingfish receiving the dosages of praziquantel (PZQ) indicated within the inlaid columns. Inlaid columns sharing the same letter are not significantly different. Data are means + SE (left-hand *y*-axis) and means − SE (right-hand *y*-axis). ▩: PZQ microcapsules; □: PZQ powder

Trial 2

Based on the food intake during the acclimation phase, fish were offered a fixed ration of 0.40% BW d^{-1} during the trial period. There was a highly significant effect of diet on ingestion (p = 0.003; Fig. 4). Those fish offered the diet surface-coated with PZQ microcapsules at 16 g kg^{-1} ate 77 ± 9% of the amount of unmedicated food eaten by the control fish, a rate significantly higher than both other medicated treatments. Those fish offered the same form of PZQ but at the higher dietary inclusion level of 25 g kg^{-1} consumed 19 ± 5% of the control ration whilst those offered PZQ powder at 16 g kg^{-1} ate only 9 ± 9% of the control ration. Ingestion rates between the 2 former diets were not significantly different.

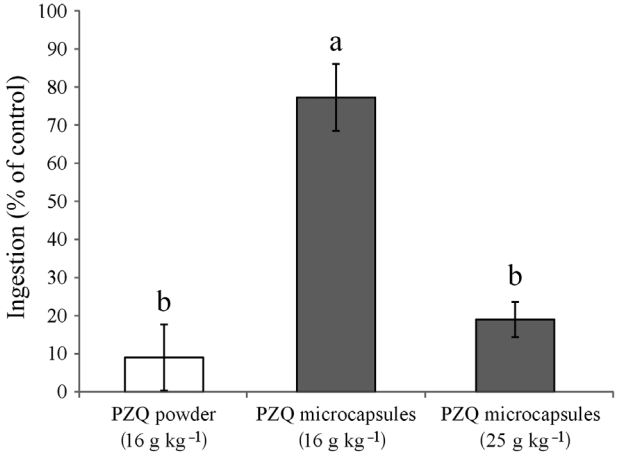

Fig. 4. Ingestion (mean ± SE) of medicated (surface-coated) feeds expressed as a percentage of the feed eaten by yellowtail kingfish receiving an unmedicated control diet. Columns sharing the same letter are not significantly different

Those fish fed the diet surface-coated with PZQ microcapsules at 16 g kg^{-1} received a dose of 45.6 ± 5.2 mg kg^{-1} d^{-1}, a dose significantly higher than that received by those fish fed the diet surface-coated with the same inclusion level of PZQ powder (5.3 ± 5.1 mg kg^{-1} d^{-1}) and those offered the diet containing PZQ microcapsules at 25 g kg^{-1} (17.4 ± 4.3 mg kg^{-1} d^{-1}).

In addition to eating less, those fish offered medicated diets took significantly longer to eat their ration compared with the unmedicated control (Fig. 5). The time taken for fish to consume the ration containing 16 g kg^{-1} of PZQ microcapsules (5.7 ± 0.7 min) was significantly less than that of those fish offered diets containing 16 g kg^{-1} of PZQ powder (8.2 ± 0.1 min) and 25 g kg^{-1} of PZQ microcapsules (7.8 ± 0.2 min), which did not differ from each other.

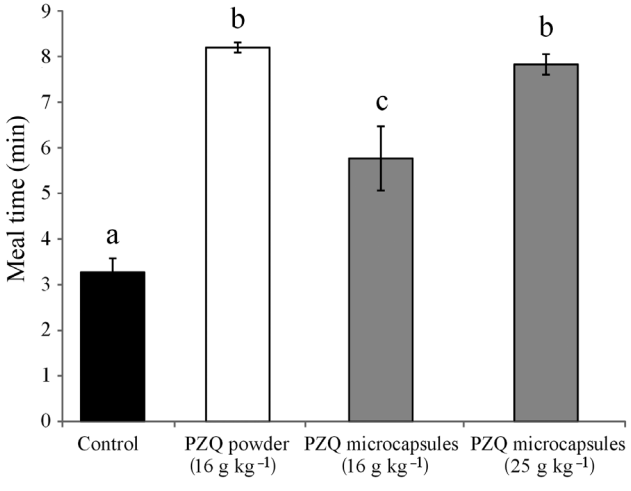

Fig. 5. Average time taken by yellowtail kingfish to consume a meal containing different types and inclusion levels of praziquantel (PZQ). Data are means ± SE. Columns sharing the same letter are not significantly different

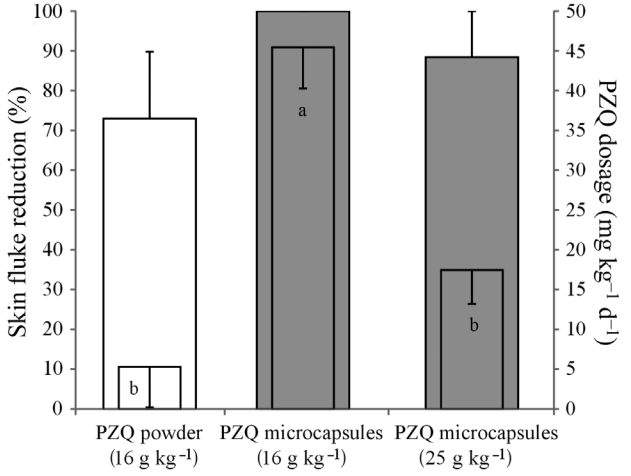

Fig. 6. Percentage reduction in skin flukes (mean + SE) relative to the control treatment in yellowtail kingfish receiving the dosages of praziquantel (PZQ) indicated within the inlaid columns. Inlaid columns sharing the same letter are not significantly different. Data are means + SE (left-hand y-axis) and means – SE (right-hand *y*-axis)

In this trial, fish were infested (naturally) only with skin flukes. Fish in the control treatment had 89 ± 11% prevalence and 2.9 ± 1.1 flukes per fish, significantly more than those in the treatment fed 16 g kg^{-1} of PZQ microcapsules, which had no flukes (0 ± 0 flukes per fish; p = 0.04). The 100% efficacy in the latter treatment was not significantly different to the 73 ± 17% reduction in the 16 g kg^{-1} PZQ powder treatment or the 88 ± 12% reduction in the 25 g kg^{-1} PZQ microcapsule treatment (Fig. 6).

The relationship between PZQ intake and plasma PZQ is shown in Fig. 7. The actual amount of PZQ ingested by fish fed the diet containing PZQ microcapsules at 16 g kg^{-1} (47 ± 9 mg PZQ kg^{-1} of fish bio-

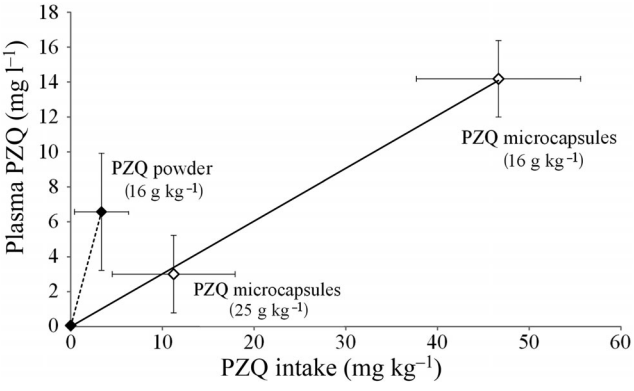

Fig. 7. Relationship between actual praziquantel (PZQ) ingested by yellowtail kingfish 2 h before blood sampling and plasma PZQ concentration. Data are means ± SE

mass) was significantly higher than that of those fish fed the same form of PZQ at 25 g kg^{-1} (11 ± 7 mg kg^{-1}; p = 0.03) due to the aforementioned significant reduction in food intake in the latter treatment. Fig. 7 also shows that the blood plasma concentration of PZQ in fish fed PZQ powder at 16 g kg^{-1} (6.6 ± 3.3 mg l^{-1}) was much higher than in those fish fed the diet coated with 25 g kg^{-1} of PZQ microcapsules (3.0 ± 2.2 mg l^{-1}), despite fish in the former treatment actually consuming much less PZQ than the latter (3.4 ± 2.9 vs. 11.3 ± 6.7 mg kg^{-1}).

DISCUSSION

The results of this study demonstrate that PZQ form, application method and dietary inclusion level influence diet palatability in large *Seriola lalandii* and efficacy against skin and gill flukes in this species.

It was hypothesised that incorporating PZQ into the mash prior to extrusion may improve the palatability of the PZQ-containing diets by minimising the amount of PZQ on the surface of the diet and therefore in direct contact with the fish's taste buds. This hypothesis was also put forward by Williams et al. (2007) as a method worthy of investigation; however, our results demonstrated no clear benefit of this application method. Whilst Williams et al. (2007) cautioned that the pressure and temperatures of the extrusion process may reduce the activity of PZQ, the fact that we saw equal fluke reduction in surface-coated and extruded diets suggests that no significant reduction in activity occurred. Furthermore, Sulieman et al. (2004) demonstrated that PZQ is highly stable against thermal decomposition. It did appear, however, that the high pressure of the extrusion process (100 PSI) caused damage to the microcapsules. Whilst incorporating PZQ into the mash prior to extrusion would theoretically enable feed manufacturers to produce medicated feeds for industry, this would be difficult to achieve in practice, due to the problems associated with carry-over and cross-contamination between medicated and non-medicated batches of feed (Daniel 2009). On-farm preparation, in contrast, has the advantage of allowing greater flexibility for adjusting dietary inclusion levels to closely match the dose rates required for different-sized fish. The benefits of this approach are outlined in further detail below.

Gelatine was used to adhere the PZQ to the exterior of the surface-coated diets, and it is possible that the gelatine also contributed to masking the bitter flavour of the PZQ. In a study by Williams et al. (2007) diet rejection was observed in *S. lalandi* offered diets surface-coated with PZQ powder and fish oil. Although the authors were unable to quantify the level of diet rejection (as the study was conducted in sea cages), the fact they achieved a lower level of fluke reduction than the present study and observed diet rejection even at the lowest dietary inclusion level of 3.8 g kg^{-1} (calculated from data on intake rate and dose) suggests that the gelatine coating used in the present study did assist in flavour masking. The mash diets in the present study were not gelatine-coated, and it is therefore possible that their palatability may be further improved with such coating. An alternative hypothesis for the difference in palatability between this study and that of Williams et al. (2007) may be due to the difference in fish size. Whilst there appear to be no studies in fish investigating the effect of age on taste sensitivity, it is well documented in mammals that sensitivity (including sensitivity to bitterness) decreases with age (Glanville et al. 1964, Mojet et al. 2001, Yamaguchi et al. 2001). It is therefore possible that the large fish used in the present study were less sensitive to the taste of the PZQ than the 320 g fish used in the study by Williams et al. (2007).

Whilst there is evidence in mammals that the *R*-(−) enantiomer of PZQ is less bitter and more efficacious against cestodes and trematodes than the *S*-(+) enantiomer (Oppel 2008, Meyer et al. 2009), both forms of PZQ used in both trials in the present study were confirmed to be racemic, and the differences in efficacy and palatability between the 2 treatments cannot, therefore, be attributed to differences in their enantiomeric ratios. Furthermore, given the very slow feeding protocol we employed together with the tank hydrodynamics used for rapid waste removal, we suggest that any leaching of PZQ from uneaten food pellets would have been negligible and would not have contributed to fluke removal.

Our data show that palatability of medicated diets is affected by the dietary inclusion level of PZQ (g PZQ kg^{-1} diet). The palatability of the diet surface-coated with PZQ microcapsules at 8 g kg^{-1} was equal to the control and superior to the diet surface-coated with the same inclusion level of PZQ powder. Increasing the active dietary inclusion level of PZQ to 16 g kg^{-1} reduced diet palatability in both the powder and microcapsule treatments (as evidenced by a reduction in both the amount of diet eaten and the time taken to consume this ration); however, the reduction in palatability in the latter treatment was far less than for the former. Palatability of the diet containing microcapsules at 25 g kg^{-1} was very poor.

These data demonstrate that whilst the microcapsules improve the palatability of PZQ, they do not completely eliminate the bitter flavour and palatability issues remain at high dietary inclusion levels.

Dose rates of PZQ are expressed as milligrams of PZQ per kilogram of fish body weight per day (mg kg^{-1} d^{-1}), and our rationale for testing different dietary inclusion levels was to enable effective dose rates to be achieved across a wide range of fish sizes in different water temperatures. Food intake decreases (on a percentage body weight basis) with increasing fish size and with decreasing water temperature, necessitating an increase in dietary inclusion levels of PZQ as fish grow and water cools in order to achieve the same dose rate. Furthermore, the removal of skin flukes, which are epithelial grazers, requires a higher dose of PZQ than for blood-sucking gill flukes to ensure adequate concentrations of the medication are transferred to the mucous and subsequently to the flukes (Tubbs & Tingle 2006). Assuming an equal sensitivity to the bitterness of PZQ across fish sizes, large fish in cool water infected with skin flukes therefore represent the greatest challenge in terms of achieving effective doses. The recommended dose rate for treating the skin fluke *Neobenedenia girellae* in yellowtail *Seriola quinqueradiata*, for example, is 150 mg kg^{-1} d^{-1} for 3 d (Okabe 2000, cited in Whittington 2012). The dietary inclusion level of 25 g kg^{-1} tested in the present study was selected to achieve this dose rate in large kingfish in cool water based on feed tables published by Masumoto (2002). The fact that we experienced a highly significant reduction in food intake at this dietary inclusion level, even with PZQ microcapsules, demonstrates that such dose rates cannot be achieved with this dietary inclusion level of racemic PZQ. Our second trial, however, demonstrated that removal of skin flukes can be achieved at a lower dietary inclusion level of 16 g kg^{-1}. This dietary inclusion level resulted in an effective dose rate in these fish of 47 mg kg^{-1} d^{-1}, much lower than the level of 150 mg kg^{-1} d^{-1} suggested by Okabe (2000). The success of our regime is likely due to the fact we fed the medicated diet for 7 d rather than the 3 d recommended by Okabe (2000). This is supported by the results of Hirazawa et al. (2004), who showed that feeding PZQ at 40 mg kg^{-1} d^{-1} for 11 d was more effective in eliminating *N. girellae* from spotted halibut *Verasper variegatus* than feeding at 150 mg kg^{-1} d^{-1} for 3 d. The authors attributed the poor performance of the latter treatment regime to the decreased appetite of the fish, which received a feed with a dietary inclusion level of 15 g kg^{-1} to achieve this dose rate compared with those given 4 g kg^{-1} to achieve the lower dose rate. Likewise, Williams et al. (2007) achieved greater fluke reduction when feeding PZQ at 50 and 75 mg kg^{-1} d^{-1} for 6 d than at 100 and 150 mg kg^{-1} d^{-1} for 3 d in *S. lalandi*.

Few studies have investigated the palatability of diets containing the highest inclusion levels tested in the present study. Tojo & Santamarina (1998) fed a diet with a very high PZQ inclusion level of 40 g kg^{-1} to juvenile rainbow trout *Oncorhynchus mykiss*. Although the authors did not report any palatability issues, elimination of *Gyrodactylus* sp. was poor despite this inclusion level delivering a theoretical dose of 800 mg kg^{-1} d^{-1}, suggesting that the diet was not well ingested. Likewise, Kim & Cho (2000) did not report any palatability issues when feeding a diet containing 20 g kg^{-1} PZQ to rockfish *Sebastes schlegeli*, yet elimination of *Microcotyle sebastis* was again poor despite this inclusion level delivering a theoretical dose of 200 mg kg^{-1} d^{-1}.

Despite a significant reduction in food intake by fish fed the diet surface-coated with PZQ powder at 8 g kg^{-1} relative to those offered the same inclusion level of PZQ microcapsules, the former fish exhibited complete gill fluke elimination whilst the latter did not. These data suggest that the coating of the microcapsules designed to mask the bitter flavour may not be completely digested by the fish, and hence the bioavailability of the PZQ within these microcapsules may be less than for PZQ powder. The blood results from Trial 2 showing higher plasma PZQ in fish fed PZQ powder than those fed PZQ microcapsules, despite the former consuming less PZQ, supports this hypothesis.

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