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The influence of the dietary inclusion of the alkaloid gramine, on rainbow trout (*Oncorhynchus mykiss*) growth, feed utilisation and gastrointestinal histology

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Abstract

This study examined the influence of the alkaloid gramine, when included in diets for rainbow trout. Quinolizidine alkaloids have been suggested as a potential anti-nutritional problem with the use of lupin (*Lupinus* sp.) meals in aquaculture diets. The findings from the present study show that above a critical threshold, the alkaloid gramine does have a strong anti-palatability effect. The effect is noted at a minimum gramine concentration of 500 mg/kg of diet, though not at 100 mg/kg. A continuing strong anti-palatability response is noted at higher inclusion levels and at the highest gramine inclusion concentration examined in this study (10,000 mg/kg), insufficient feed was consumed to even supply maintenance protein and energy demands. No adaptation to concentrations of gramine was observed throughout the 6-week study. No effects on nitrogen, energy or phosphorus digestibility were seen at the 500 mg/kg inclusion concentration of gramine relative to the reference diet, although the inclusion of the yellow lupin kernel meals (both Wodjil and Teo varieties) in the diet did improve the digestibility of phosphorus. Growth, as assessed using a range of parameters including weight gain, growth rate, nutrient and energy retention of fish fed the experiment treatments was largely consistent with feed intake. Survival of fish was significantly reduced at gramine inclusion levels above 1000 mg/kg. Feed conversion ratio (FCR) and feed conversion efficiency (FCE) were also reflective of feed intake and growth levels observed of each treatment. The concentrations of the plasma thyroid hormones tri-iodothyronine (T_3) and thyroxine (T_4) of fish from each of the treatments were consistent with feed intake (including the controls) suggesting that the concentrations of these hormones are in response to feed intake, not specifically the gramine levels in the diets. However, the inclusion of the *Lupinus luteus* kernel meals resulted in a significant change in T_4 levels, with a degree of independence of the

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feed intake, suggesting that there may be another mechanism by which these meals are influencing the concentrations of this hormone. In this study, there was an increase in the density of melano-macrophage centres (MMC) with high dietary levels of gramine. However, in the absence of any histological evidence for a toxic effect, it is likely that the increased MMC densities observed in the fish fed high concentrations of gramine are associated with starvation. This study demonstrated that the lupin alkaloid gramine, can have a strong anti-nutritional effect on fish at inclusion concentrations greater than 100 mg/kg, but that its mode of action is primarily through an anti-palatability effect. It is therefore considered unlikely that alkaloid effects would be observed in diets even with 50% inclusion of kernel meals from Australian commercial *L. luteus* varieties.
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1. Introduction

It is well recognized in the aquaculture feeds industry that there is a need to reduce reliance on fish meal in aquaculture feeds (Naylor et al., 2000). Increasing the actual or prospective utilization of other protein meals in diets for aquatic species, substantial risk reduction is achieved. The use of plant protein meals as alternative protein resources has been well studied and many viable options including soybean, glutens and lupin meals have been adopted industrially (Carter and Hauler, 2000; Storebakken et al., 2000; Glencross et al., 2004). However, the introduction of anti-nutritional factors and other biologically active compounds can accompany the use of plant protein meals (Francis et al., 2001).

Anti-nutritional factors (ANF) can affect the utilization of food by an animal through several avenues, including the metabolic axis, nutrient digestibility or ingredient palatability (Refstie et al., 1998, 1999; Glencross et al., 2003a,b). Alkaloids are heterocyclic amino acid derivatives produced by plants as a chemical defence mechanism. While alkaloids are found in most legume species, they have traditionally been found in high concentrations in the seeds of plants from the *Lupinus* genus (Petterson et al., 1997; Wasilewko and Buraczewska, 1999). Notably, a variety of alkaloids are found in these seeds. In some varieties of the species *Lupinus luteus*, a major alkaloid component is gramine (Petterson, 2000). Feeding studies with kernel meals from the seeds of *L. luteus* have shown good prospect for their use in aquaculture feeds because of their high digestible protein content, although some deterioration in growth performance at high inclusion levels has been noted (Glencross et al., 2004).

Consumption of gramine at toxic levels in mice has been noted to lead to psychotropic levels of excitement and seizure. The mode of action for gramine as an ANF, or toxicity data on this compound is limited. However, mammalian effects include changes in tubules and glomeruli in the kidney, ureter and bladder, endocrine changes in spleen weight, and biochemical changes such as enzyme inhibition, induction via changes in blood or tissue levels of phosphatases (TXCYAC, 1980), although no specific data is available for any fish species. Tolerance concentrations to the inclusion of dietary gramine in other vertebrate species (rats, pigs and poultry) have been determined at; about 300 mg/kg for rats, >500 mg/kg diet for pigs and about 650 mg/kg diet for poultry (Pastuszewska et al., 2001). The effects of concentrations as low as 250 mg/kg of *Lupinus angustifolius* alkaloids have been reported in rats (Butler et al., 1996), although concentrations of alkaloids from *L. albus* were only reported to have an adverse effect at 320 mg/kg (Zdunczyk et al., 1998).

The current Australian commercial *L. luteus* variety (Wodjil) has very low gramine concentration compared to European varieties such as Teo. However, Wodjil has proven agronomically costly to produce because of the high levels of insecticide use required to deal with substantial insect infestation problems (Perry et al., 1998; Berlandier and Sweetingham, 2003). There is evidence that aphid infestation is directly related to the low inherent concentration of gramine (Risdall-Smith et al., 2004). Higher alkaloid varieties of *L. luteus*, such as Teo, have better resistance to insect infestation, but it is unclear whether the higher alkaloids will influence the usefulness of the kernel meal as an aquaculture feed ingredient.

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